1991

Study of En at the wx-844 allele: modifier of En excision, weak En, and transposition of En

Sudhansu Dash
Iowa State University

Order Number 9126187

Study of *En* at the *wx-844* allele: Modifier of *En* excision, weak *En*, and transposition of *En*

Dash, Sudhansu, Ph.D.

Iowa State University, 1991

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

Study of *En* at the *wx-844* allele: Modifier of *En* excision, weak *En*, and transposition of *En*

by

Sudhansu Dash

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Zoology and Genetics
Major: Genetics

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1991

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FLOW DIAGRAMS

LIST OF FIGURES

1. INTRODUCTION

Classical studies in genetics established that genes have fixed locations on the chromosome. McClintock (1946, 1948) discovered a class of genetic elements in maize and showed that they are capable of changing their chromosomal location. Since then, many others have been studied in maize and other organisms. Because of their mobile nature, they are variously named as transposable elements, transposons or mobile elements.

A transposon has been defined as a sequence of DNA that can excise from its original location and reinsert at another location; and the process has been termed as transposition. Transposition is acheived by the action of an enzyme, transposase coded in the sequence of the transposon.

The *Enhancer* or *En* transposable element has been studied in this dissertation. Nearly four decades of its study by Barbara McClintock and Peter A. Peterson, following the discovery of *En* by Peterson (1953) has made it one of the genetically best characterized transposons in maize.

The alternative name, *Spm* (*Suppressor-mutator*) coined by McClintock (1954) signifies its mode of action. The insertion of *En* or a deletion derivative of *En*, also known as an *I* element, within a gene blocks the expression of the gene completely or partially. Upon excision of the insert, the

gene expresses itself and is phenotypically observed as wild type sectors in a background of null or partial expression. *En* as a discrete genetic unit encodes for this excision capability and this ability of *En* to cause excision is known as its mutator or *M* function. The *I* elements lack this capability although, they can be excised if an active *En* is present in the genome providing the mutator function. The other function, the Suppressor function is evident in cases where the insertion of an *I* element allows partial gene expression. In this case the *En* element, if present in the genome, is also capable of suppressing the partial background expression, thus, manifesting its suppressor function.

The insertion of *En* at the *Wx* gene (Peterson, 1985) and the subsequent molecular isolation of the 8287 base pair long *En* transposon (Pereira et al., 1985) from the *wx-844* mutable allele made it possible to investigate the molecular nature of the *En* functions. Deletion derivatives of the *En* element defective in *En* functions can be studied genetically and molecularly to correlate its molecular features (Gierl et al., 1985; Pereira et al., 1986) to its established genetic functions. The mutability induced by *En* is subject to modifying factors (McClintock, 1958; Reddy and Peterson, 1983). An *I* element functioning as a modifier of mutability, discovered during the course of this study, also became suitable towards the study of *En* functions. The *En* containing *wx-844*

allele also made it possible to simultaneously monitor the destiny of *En* in the endosperm as well as the aleurone tissue of the maize kernel.

In this study a defective *En* element has been isolated from the *wx-m 86246X* allele, a derivative of the *wx-844* allele, and has been genetically characterized to augment molecular studies towards the understanding of *En* functions. The modifying effect of the *a-m(r) 102* allele on *En* mediated excision has been established and investigated to understand the working of the mutator function of *En*. The *wx-844* allele has also been used in combination with other *I*-element-containing mutable alleles to study the destiny of *En* following excision in somatic tissue.

2. REVIEW OF LITERATURE

2.1. Transposable Elements

2.1.1. Features of transposable elements

Transposable elements or transposons are DNA sequences that are mobile in nature. They transpose from one site to another in the genome and for this reason, are also termed as mobile elements. They have been found to occur in prokaryotes, plants and animals. They were first discovered in maize and there are 10 to 12 different transposable element systems in maize. They have distinct molecular features. All transposons studied molecularly have terminal inverted repeats (TIR) at both termini of the element. The TIR sequence is oriented in opposite directions at the termini. The number and sequence of nucleotides at the TIR is a characteristic feature of a specific transposable element. The immediate internal sequence to the TIR is usually rich in duplications and/or inversions of short sequences.

There is another distinctive feature of mobile elements and this is that they generate short target site duplication (TSD) at the site of insertion. The number of nucleotides in the TSD is also a characteristic feature of specific transposable elements although, the precise sequence of bases depends on the sequence of the site of insertion.

Active elements range in size from 4.3 kb (*Ac*) to 17 kb (*Tam1*). There is a part of the internal sequence that codes for the transposase, an enzyme necessary for transposition.

Except in some prokaryotic transposons, transposition consists of excision from the original site and subsequent insertion at a new site. Excision of an element may be precise or imprecise. Precise excision consists of removal of the transposon at both the TIR and reconstitution of the original target site. Imprecise excision is often the rule than an exception (Schwarz-Sommer et al., 1985a; Saedler and Nevers, 1985; Gierl et al., 1989; Gierl and Saedler, 1989).

2.1.2. Detection of transposable elements

Transposable elements are obvious when they interrupt gene activity. Thus, the site of insertion of a transposable element can be within the sequence of a gene that shows a visible phenotype. This insertion represents a foreign sequence within the structure of a gene and thus impedes or restricts the expression of the gene. The abolition of gene expression occurs usually due to stoppage of transcription of the gene at the inserted foreign sequence. This insertional block results in a null phenotype. Precise to near precise excision of the transposable element releases the gene from the insertional inactivation. The excision event occurs only in a few cells. The gene is then expressed in the clonal

descendants of the cell in which excision has occurred. The phenotypic consequence of excision is generally wild type sectors in a background of null expression. In general, wild type sectors in a null phenotypic background indicates the presence of transposable elements; although other cases of similar variegation are known not to be due to transposable elements.

The allele carrying the insert inside the gene is called a mutable allele or unstable allele since it somatically mutates or reverts to wild type usually from a recessive form of expression. If the excision event occurs in a tissue giving rise to the germinal tissue, the wild type revertants can be recovered in the gametes and hence, in the next generation among the progeny. These germinal events are helpful in further genetic tests. Most transposable elements have been detected when resident within a gene and effecting a mutable phenotype (Emerson, 1917; Rhoades, 1938; Peterson, 1953; McClintock, 1954; Gonella and Peterson, 1977; Robertson, 1978; Friedemann and Peterson, 1982; Schnable and Peterson, 1986).

Because of their unique structure, some transposable elements are known to cause chromosome breakage at or near their site of insertion, for instance, the *Ds* element (McClintock, 1946, 1947, 1948). Chromosome breakage causes

loss of distal markers and in a suitable genotype the loss of markers can be observed as variegation.

A few transposable elements have been detected solely by molecular means. A DNA sequence that has the features of the transposable elements (e.g., TIR, TSD, and subterminal region rich in duplications and inversions) is usually assumed to be a transposon although its transposing ability may or may not be known. The maize *cin1* element has been detected solely by its structural characteristics (Shepherd et al., 1982).

2.2. Maize Transposable Elements

Transposable elements, as they are known today, were first discovered in maize by Barbara McClintock (1946, 1948, 1950a, 1950b, 1951, 1952). The transposable elements of maize have been well characterized at the genetic level (reviewed by Fincham and Sastry, 1974; Fedoroff, 1983, 1989; Freeling, 1984; Nevers et al., 1986; Döring and Starlinger, 1986; Peterson, 1986, 1987; Schwarz-Sommer and Saedler, 1988; Gierl et al., 1989; Gierl and Saedler, 1989).

2.2.1. Regulatory and receptor elements

In maize two classes of transposable elements are recognized, the autonomous (Fedoroff, 1983) or the regulatory element (McClintock, 1961b) and the non-autonomous (Fedoroff, 1983) or the receptor element (Peterson, 1965; Fincham and Sastry, 1974). The autonomous elements code for the function(s) necessary for their own transposition and also for the transposition of the corresponding receptors, i.e., they are autonomously transposition competent. The non-autonomous elements can not transpose by themselves; they need the transacting functions of the corresponding regulators for transposition. The mutable allele carrying the autonomous element is called an autonomously mutable allele since its mutability is conditioned by the resident autonomously transposition competent element. The mutable allele carrying the receptor element is termed as a non-autonomously mutable allele. It shows mutability only in the presence of the regulatory element elsewhere in the genome.

2.2.2. Transposable element systems in maize

The regulator and the receptor elements show very specific interaction. Based on their specific interaction a transposable element system is defined (detailed review by Peterson, 1987). Not all receptors respond to the transactive functions of all regulatory elements. Interaction

between regulator and receptors takes place within and identifies a system. A system is thus comprised of regulatory elements and their corresponding receptor elements. Based on the above criteria more than a dozen transposable element systems have been defined. These include among them, *Ac-Ds* (McClintock, 1947, 1951), *En(Spm)-I(dSpm)* (Peterson, 1953), *Dt-rDt* (Rhoades, 1938), *Fcu-rcu* (Gonella and Peterson, 1978), *Uq-ruq* (Friedemann and Peterson, 1982), and *Cy-rcy* (Schnable and Peterson, 1986).

Of all the transposable element systems in maize only *Ac-Ds*, *En-I*, and the *Mu* elements have been molecularly characterized in detail. Most of the molecular work has been done with the *Ac* and the *En* transposon (reviewed in Gierl et al., 1989). The receptor elements in case of *Ac* and *En* have been found to be deletion derivatives of the regulatory element. The deletion adversely affects the expression of the transposase and this explains why the receptor elements are not autonomously transposition competent.

2.3. The *En* Transposable Element

2.3.1. Discovery to molecular isolation

Study of mutability at the *Pg* locus led to the discovery of *En* (Peterson, 1953, 1960). Later mutable alleles at the *A* locus containing the *En* transposable element were isolated

(Peterson, 1957, 1961). Its location in an anthocyanin gene was used advantageously to make it one of the genetically best characterized transposons in maize. McClintock's *Spm* element studied with the *a-m1* (McClintock, 1951, 1954, 1955) mutable allele was later found to belong to the same system (Peterson, 1965). Later, Peterson (1985) also isolated the *En* element at the *Wx* locus. Availability of the *Wx* gene clone made it possible to molecularly isolate (Pereira et al., 1985) the *En* transposon from the *wx-844* allele. Molecular characterization (Pereira et al., 1986) of this allele revealed that the *En* is a 8287 bp long transposon and has a complex structure.

2.3.2. Regulators and receptors of the *En-I* system

Peterson defined the *En-I* system while studying the pale green mutable (*pg-m*) allele (Peterson, 1953, 1960). He termed the regulator element as *En* for Enhancer and the receptor element as *I* for Inhibitor. *En* is autonomously transposition competent. The receptor *I* needs the trans-active functions of *En* for excision.

McClintock (1954, 1955, 1965b) has independently defined the *a-m1* and *Spm* system. Later Peterson (1965) established that *Spm* is genetically homologous to the regulator *En* and the receptor at *a-m1* homologous to *I*. Fedoroff (1983) has

coined the term *dSpm* for the receptors of *Spm*. Thus the equivalent terms are *En* = *Spm* and *I* = *dSpm*.

2.3.2.1. The mutability due to insertion of the regulator, *En* The autonomously mutable alleles of the *En-I* system contains the *En* inserted within the gene. The insertion of *En* within a gene results in the complete block of gene expression. Excision of the *En* relieves the gene from the steric block and the gene is expressed if excision is precise or near precise. This phenomenon is observed both somatically and germinally. Somatically, this is observed as wild type sectors in a null phenotypic background. If the insertion is in a gene controlling anthocyanin pigment formation, e.g., *A* , the result is that of sectors of pigmented cells in a background of non-pigmented aleurone. This phenotype is alternatively described as colored spots or sectors in a colorless background or simply, spotted or sectored. In this example such a mutable allele is named as an *a-m* allele for *a-mutable*, i.e., an allele showing mutability at the *A* locus.

Germinal excision also occurs. Three phenotypic consequences of germinal excision are described here. Near precise excision events lead to recovery of a gamete containing the gene whose expression can be comparable to that of the wild type allele. In some instances less than wild type

expression is also observed. The alleles generated from the mutable alleles via excision are called revertants. Instances of reversion to null alleles are also observed, e.g., *a-m(Au)* and *a-m(papu)*.

The null alleles can be classified into two groups depending on their responsiveness to *En* present elsewhere in the genome. Some of them show mutability in the presence of *En* and are called responsive alleles. Others remain stable in their expression in the presence or absence of *En*. They are termed non-responsive alleles.

A null allele if it responds to the presence of *En* by showing mutability is called an *m(r)* allele for example *a-m(r)*, *c-m(r)*, etc. depending on the locus where the *m(r)* allele is recovered. The *m(r)* stands for mutable and responsive. A derivative allele showing intermediate expression is called pale *m(r)* if responsive to *En*. Thus, non-autonomously mutable alleles can be derived from the autonomous ones at the same locus. An example is the *a-m(r)* allele that is derived from the autonomously mutable *a-m(dense)* allele (Peterson, 1961).

In other cases of excision of *En* from the locus, null alleles have been recovered that are not responsive to the presence of *En*. They remain stable in their null expression irrespective of the presence or absence of *En*. They are called *nr* alleles for non-responsiveness. For example non-

responsive alleles recovered from the *a-m(papu)* allele are termed *a-nr* (Nowick and Peterson, 1981; Peterson, 1970).

The presence of *En* elsewhere in the genome, i.e., not inside a gene with a visible phenotype, is not detectable due its own excision. Detection of such floating *En*s is possible in the presence of a non-autonomously mutable allele whose mutability is caused by the necessary functions provided by the *En* present anywhere in the genome. Therefore, non-autonomously mutable alleles can be used as reporter alleles (Peterson, 1987) to detect the presence of *En*. Similarly, when a mutable allele is present in the heterozygous condition with a wild type allele, the mutable phenotype is also not detectable. Although excision occurs, it is masked by the uniform expression of the wild type allele.

Autonomously mutable alleles arise by transposition of *En* from other locations of the genome to within a wild type allele of a gene (Peterson, 1978).

Another class of autonomously mutable alleles shows a lower mutability rate. They have been named *En*-weak or *En*-low (*Spm-w* by McClintock, 1956, 1957, 1961a, 1963, 1965b; *En'* by Peterson, 1966, 1976a). McClintock (1965b) isolated a weak element at the *a-m2* allele and described that it differs from the standard elements primarily by its ability to cause excision at the *a-m1* allele.

2.3.2.2. The mutability due to insertion of the receptor, *I* The non-autonomously mutable alleles contain the receptor element of the *En-I* system inserted within the gene. The receptor element of the *En-I* system is called an *I* element for the Inhibitor. The result of insertion of an *I* element within a gene has been observed to affect the expression of the gene in one of the two ways mentioned below. In one type of insertion, gene expression is completely blocked showing a null phenotype in the absence of *En*. Presence of *En* causes the excision of the *I* element and is observed as spotting or sectoring. A typical example is the *a-m(r) 102* allele that shows a colorless aleurone in the absence of *En* and heavily spotted phenotype in the presence of *En* (Peterson, 1961).

A second type of insertion does not block gene expression completely, but instead allows partial to full expression of the gene (background expression). As a result, these alleles show a lower than (*a-m1 5719*) to full wild type (*a2-m1* state II) phenotype in the absence of an active *En* in the genome (McClintock, 1954, 1958). Presence of *En* in the genome causes excision of the *I* resulting in wild type sectors in a null background. The background expression that is seen in the absence of *En* is not seen in the presence of *En*. The suppression of this background expression is called suppressor or the *S* function of *En*. The *En* mediated excision

of the *I*, causing spots of wild type expression is called the mutator or the *M* function of *En*.

Another type of insertion, and the only known thus far in the *En* system, causes a null expression in the absence of *En*. The presence of *En* causes spots due to excision in addition to a background of low expression of the gene. This *I* insertion is found in the case of the *a-m2* alleles where the *I* insertion is in the promotor of the *A* gene (Schwarz-Sommer et al., 1987), controlling production of anthocyanin pigment. In the absence of *En* this allele shows a colorless phenotype. When *En* is present, spots are seen in a pale colored background. The pale colored background is due to expression of the *A* gene in the presence of *En*, i.e., the allele is said to be co-expressed in the presence of *En*. This is called the co-expression or *coex* function of *En* (McClintock, 1965a, 1965b, 1968; Reddy and Peterson, 1985).

These three functions of *En* are elicited depending on which reporter allele is present (Section 3.5). The first category (e.g., *a-m(r) 102*) elicits only the *M* function of *En*. The second category (e.g., *a-m1 5719*) elicits both *S* and *M* functions of *En*. The last category (e.g., *a-m2 8004*, *a-m2 4412*) shows the *coex* and the *M* response to *En*. These *I* containing mutable alleles can also be used to detect the presence of *En* since they show distinct phenotypes in the presence and absence of *En*. For this reason, they are called

reporter alleles. Alteration in one of the three functions of *En* can be detected using an appropriate reporter allele.

Non-autonomously mutable alleles arise in two ways: From the autonomously mutable alleles as mentioned before with the *m(r)* alleles and from transposition of an *I* element into a gene as is the case with the autonomously mutable alleles.

2.4. Molecular Structure of *En* and *I* Elements

2.4.1. Molecular structure of *En*

The *En* transposable element was cloned after it was isolated at the *Wx* locus. The following cross was set up to recover an event where *En* has transposed to the *Wx* locus. *Wx/Wx En* X *wx/wx*. Approximately 2.5 million gametes were screened for the wx-mutable phenotype. One, out of four such wx-mutable isolates was proved to be autonomously wx-mutable and contained *En*. This allele was named *wx-844* (Peterson, 1985). In this allele the *En* transposon was found to be in the seventh intron of the *Wx* gene. The *En* molecularly isolated and characterized (Pereira et al., 1985) from this locus is named as *En1*.

The *En1* is 8287 bp long with perfect 13bp TIR. The sequence of the TIR is not repeated in the rest of the sequence. At the site of insertion a 3bp TSD is generated. The

ends of *En1* are highly structured. A 13-mer nucleotide motif is repeated eight times in the left and ten times in the right end in direct or inverse orientation. The homology between the two ends is restricted to ~200 bp of the left terminus and 300 bp of the right terminus. The sequence organization of the termini makes it possible to pair the left and right ends in a stem and loop structure. Alternatively stem and loop structure can be formed within each end (Gierl et al., 1985; Pereira et al., 1985).

The *En* elements cloned from the mutable alleles of the *A* locus (O'Reilly et al., 1985), and two from the *C* locus (Paz-Ares et al., 1986) are identical in structure to *En1* at the level of restriction and heteroduplex analysis. An *Spm* element was also sequenced from the *a-m2* allele and was shown to differ from the *En1* sequence in only eight positions (Masson et al., 1987). The high homology is as expected from the genetic similarity of the two elements (Peterson, 1965).

Two weak *En* elements have also been molecularly characterized. The *En2* (*wx-m 86246X*), found and described in this study contains a deletion of 1126 bp in the middle of the element (Gierl et al., 1988a). The *Spm-w* element at the *a-m2* allele (McClintock, 1963) also contains a very similar deletion of 1.6 kb (Banks et al., 1985). Both the elements have almost identical 3' ends.

2.4.2. The structure of *I* elements

The *I* elements in the *En-I* system have been found to be deletion derivatives of the autonomous element, *En*. This is true for all the *I* elements molecularly characterized so far (Gierl et al., 1985; Masson et al., 1987; Tacke et al., 1986; Schiefelbein et al., 1988). The deletion affects the coding of the necessary functions for transposition and explains why an autonomous element is necessary for excision of the *I* elements. The ends of the *I* elements, as is true for *En*, are necessary for the protein products of *En* to act on and to affect excision and transposition.

There is a good correlation between the amount of sequence affected by the deletion and contributing to the stem and loop structure at the termini and the efficacy with which the *I* element excises. An intact stem and loop structure seems to be necessary for appropriate excision.

Two series *I* elements exemplify the above situation clearly. One series at the *A* locus (Tacke et al., 1986) consists of the mutable alleles *a-m1 6078*, *a-m1 5719A-1*, and *a-m1 1112*. The later two have been shown to be deletion derivatives of *a-m1 6078*. The *a-m1 6078* allele has the complete loop and stem structure and expectedly also shows a high rate of excision. The *a-m1 5719* allele has a deletion that affects a small part of the stem and loop structure at the left side, consequently, the excision rate observed with

this allele is drastically reduced compared to that observed in the case of *a-m1 5719*. The *a-m1 1112* has the deletion that affects approximately half of the stem and loop structure on the left side. The excision rate shown by this allele is also lower than that of *a-m1 5719* allele. A similar pattern of excision frequency and intactness of the stem and loop structure is also observed with a series of deletion derivatives isolated from the *bz-m13* allele (Schiefelbein et al., 1988).

2.4.3. Transcription and Gene Structure of *En*

A 2.5 kb major transcript is found in *En* containing lines (Pereira et al., 1986). Several minor transcripts of sizes 1.1 kb, 1.7 kb, and 6 kb can also be observed. Cloning and sequencing the cDNA corresponding to the 2.5 kb major transcript showed that it spans the entire length of the *En* element (Pereira et al., 1986) (Figure 2.1). The transcription is initiated from a promotor (P in Figure 2.1) at the left end of the element. The minor transcripts have the same direction of transcription. cDNA analysis of the 2.5 kb transcript has established the structure of the corresponding gene, which has been termed *tnpA* (Cuypers et al., 1988) (Figure 2.1). This gene has 11 exons. The gene sequence is bordered at its 5' and 3' extremities by the short repeat sequences which compose the highly structured termini of *En*.

The trans-active functions which recognize and interact with the termini probably influence the transcriptional activity of the *tnpA* promotor which overlaps the repeat sequences of the left terminus. The potential control sequences upstream of the transcription start of *tnpA* which include the -30 TATGAA sequence, lie within the stem and loop structure. Two possible translation start sites are at the beginning of the second exon (Pereira et al., 1986).

TnpA codes for a putative protein of 68 kd. The termination codon present in the eleventh exon is followed by the polyadenylation site. A possible polyadenylation signal (AATATG) precedes the polyadenylation site. All exon-intron boundaries obey the 'GT-AG' rule (Breathnach and Chambon, 1981). The first exon compared to the rest of the *En* is high in GC content. Frequent occurrence of CpG residues may contribute to the regulation of *En* activity through methylation in this region.

One unusual feature of the *tnpA* is its exceptionally large, 4434 nucleotide long first intron (Figure 2.1). This long intron contains two non-overlapping open reading frames (ORFs). The ORF1 is 2714 nucleotides long and the smaller ORF2 is 761 nucleotides long. A possible translation start site is located in the beginning of ORF1 (Figure 2.1). At a position within the ORF2 the ORFs can be spliced to the second exon without disturbing the open reading frame. The

significance of the ORFs can be realized in the longer transcripts observed which hybridize to the *En* fragments containing sequences from the ORFs (Pereira et al., 1986; Gierl et al., 1988a, 1988b; Cuypers et al., 1988).

2.4.4. *En* functions

2.4.4.1. *En* functions at the phenotypic level

The various non-autonomously mutable alleles respond to the presence *En* in different ways. All of them show mutability, i.e., excision of the *I* element inserted in the gene. This effect of *En* has been termed as the mutator or *M* function of *En*. The name suggests that mutation of a null (amorph) to wild type phenotype is induced in the presence of *En*. The null or sometimes hypomorphic phenotype is due to the insertion of the *I* element and the wild type phenotype is due to the near-precise excision of the *I* element. This event occurs both somatically and in the germinal tissue.

Some of the non-autonomously mutable alleles do not show a null (amorph) phenotype in the absence of *En*. They show a hypomorphic phenotype, instead. For example, the alleles *a-m1 5719*, *a-m1 1112*, *c2-m2*, and *bz-m13*, all of which are in the anthocyanin pathway show a pale pigmentation in the absence of *En*. One extreme example is the *a2-m1* allele which shows a wild type pigmentation in the absence of *En*. This is

the background expression. In the presence of an *En* element in the genome this background expression is suppressed and therefore, not seen. These kernels show a null or colorless background and spots or sectors due to excision. The disappearance of the background expression in the presence of *En* is called the suppressor effect or the *S* function of *En*, resulting from the suppression of the background expression. On the basis of these two functions, suppressor and mutator, McClintock termed the regulatory element as *Spm*: *Sp* for suppressor and *m* for mutator. She described *Spm* having two components of action; component 1 and component 2 (McClintock, 1965b, 1971). Component 1 is responsible for the suppressor effect and component 2 for the mutator effect.

In the case of another non-autonomously mutable allele, the a-*m2* alleles, spots are seen in a background of pale coloration. The allele shows a colorless phenotype in the absence of the *En* element. Various degrees of pale coloration of background expression are observed only in the presence of the *En*. Reddy and Peterson (1985) have termed the expression of the *A* gene in the presence of *En* as the co-expression effect or the *coex* function of *En*. McClintock (1965, 1971) has described it as the effect of the component 1 of *Spm*.

Another function of *En* has been defined by Nevers and Saedler (1977) and this is the effect of an active *En* to

activate an inactive *En*. They have termed it as the *A* function of *En*. Again McClintock (1965, 1971) has described the same effect as the function of the component 1 of *Spm*.

In a genetic sense, four functions have been defined, viz., *S*, *M*, *coex*, and *A*. But the molecular findings indicate that they are the expression of only two components of the *En* or *Spm* element. All these functions are not elicited with all the mutable alleles. Only certain reporter alleles can elicit certain functions. Therefore to study a specific *En* function, specific reporter alleles are used.

McClintock has also described that component 1 of *Spm* is necessary for the activity of the component 2, that is, component 2 cannot function unless component 1 is active.

2.4.4.2. *En* functions at molecular level The major transcript observed in active *En* containing lines is the 2.5 kb defining the *tnpA* gene product. *TnpA* codes for a putative protein of 68 kd. The amino acid sequence of the *tnpA* protein predicted from the cDNA sequence shows homology to the transposase of the P element in *Drosophila*. The five amino acid homology found in the amino terminal half of the *tnpA* protein represents a helix-turn-helix region of the P element transposase. This region is implicated as a DNA binding domain (Rio et al., 1986).

Gierl et al. (1988a, 1988b), established that the *tnpA* product is a DNA binding protein. The *tnpA* protein recognizes a 12 bp motif: CCGACACTCTTA, closed versions of this sequence are scattered at either end of the *En* element. Six such binding sites are present in the left end and eight are present in the right end of *En*. Their binding assay studies show that binding is stronger to a DNA fragment when more binding motifs are present compared to binding to a fragment containing only one binding motif. The binding is also reduced when the CG or the CNG sites of the motifs are methylated.

The discovery of the *tnpA* product as a binding protein to specific motifs present at the end of the *En* element has suggested a model for the *S* or suppressor function of *En*. Some alleles carrying an *I* insertion do not show complete abolition of gene activity. The residual gene expression of these alleles is attributed to transcription of the entire unit containing the gene and the inserted element, followed by processing of the primary transcript such that most of the element sequence is removed (Gierl et al., 1985; Tacke et al., 1986; Kim et al., 1987; Schiefelbein et al., 1988). Presence of *En* in the genome abolishes the residual gene activity of these suppressible alleles. Gierl et al. (1985), observed in case of the *wx-m8* allele (a 2.2 kb *I* element insertion in the *wx* gene) that transcripts initiated from the

wx gene promoter extend into the *I* element and these chimeric transcripts are absent in the presence of an *En* element. They speculated that *En* encoded products bind to the ends of the element and suppress the appearance of the transcripts that extend into the *I* element. Schwarz-Sommer et al. (1985b), explained using the same model the suppression of the residual pale coloration of the *a-m1 5719* allele in the presence of *En*. The binding of *tnpA* product to the ends of the *En* element validates the above model. The *tnpA* product was confirmed to be the suppressor function of *En* by reconstituting a suppressible system in tobacco (Grant et al., 1990).

The *M* or mutator function of *En* is responsible for the excision of the *En* and the *I* elements from their site of insertion followed by reinsertion at another site. The ends of the element, both the TIR and the subterminal regions are the site of action of the *M* function of *En*. This evidence comes from the *I* elements having various extents of deletion in the subterminal region. Increased extent of deletion in the subterminal region adversely affects the excision frequency (Schwarz-Sommer et el., 1985b; Schiefelbein et al., 1985, 1988; Tacke et al., 1986; Masson et al., 1987). The 13 bp TIR is also important for excision. Even minor changes in the nucleotides drastically reduce the excision frequency (Schiefelbein et al., 1988). Similar consequences have been

observed in the related *Tam1* element of *Antirrhinum* (Hehl et al., 1987).

The subterminal 12 bp motifs repeated at both ends of the element bind to the *tnpA* product, however, the 13 bp TIR sequences do not interact with the *tnpA* product (Gierl et al., 1988b). It is likely that the *tnpA* protein is necessary for excision to occur but not the sole agent in mediating the excision function.

A second *En* encoded product is implicated to mediate the excision and transposition of the elements. One of the minor transcripts observed in northern experiments is 6 kb long. This has been shown to hybridize with the ORF sequences of *En* (Pereira et al., 1986). The expression of the ORF1 sequences is also implied in the transposase function. The ORF1 of *En* shares significant amino acid homology with the related *Tam1* transposon of *Antirrhinum* (Sommer et al., 1988) and the *Tgm1* transposon of Soybean (Rhodes and Vodkin, 1988). In transgenic tobacco, excision of *En* is also accompanied by the expression of the 6 kb transcript (Pereira and Saedler, 1989). The 6 kb message is absent in transgenic tobacco lines lacking transpositional activity. In transgenic potato plants showing *En* transposition (Frey et al., 1989), the 6 kb transcript is also expressed. This supports the idea that the protein product specified by the 6 kb message is necessary for the transposition function. The *M* function of *En* is

associated with transposition (i.e., excision and reinsertion) and therefor is likely coded by *tnpB* (now termed *tnpD*) which expresses the ORF sequences in the 6 kb message (Gierl et al., 1988b). The 6 kb transcript is most likely a product of alternate splicing from a larger primary transcript in which the ORF sequences are spliced into the end of exon1 and the start of exon2.

The suppressor function of *En* or McClintock's component 1 and the mutator function of *En* or McClintock's component 1 are the *tnpA* and *tnpD* at the molecular level. The co-expression function and the activator function of *En* are the effects of the component 1 and therefore expression of the *tnpA* product of *En*.

2.5. Modifiers of *En*

Modifiers are described as factors or elements that alter the mutability pattern of a mutable allele. McClintock has described a type of modifier that enhances the rate of mutability. In the presence of a weak *Spm* and the modifier the frequency of excision increases at the *a-m1* reporter allele. The modifier also enhances mutability of low spotting *a-m1* alleles in the presence of a standard *Spm* (McClintock, 1956).

Peterson (1976a) has described a negative modifier of *En*, called *Rst* for restrainer. Similar modifiers named *En-malt* have been characterized by Reddy and Peterson (1983). They are suggested to have modified *M* action and are derived from the *En* element.

2.6. Transposition of *En*

Transposition of *En* from a locus to other sites and to a locus from an unknown site is well documented. *En* was originally identified at the *pg* locus on chromosome 3. From this *pg-m* allele independently segregating *En*s have been isolated (Peterson, 1953). The isolation of the autonomously mutable *a-m* allele (Peterson, 1961) is also an example transposition of *En* to a locus from an unknown location in the genome. Similarly Peterson (1978, 1985) has also isolated *En*s at the *a2*, *c*, and *wx* loci as a result of transposition of *En* from an unidentified location to these loci.

Extensive studies of transposition of *En* have been done from the *a* locus (Peterson, 1970; Nowick and Peterson, 1981; Peterson, 1987). Of all the excision events from the *a* locus, *En* was lacking in 38% of the cases. 62% of the excision events showed the presence of *En*. Of these transposed *En*s, 39 to 50 per cent showed linkage to the donor site, i.e, the *a* locus (Peterson, 1987).

Nowick and Peterson (1981) studied transposition of *En* from three autonomously *a-mutable* alleles. Of the 200 transposition events studied, *En* was found in all regions of chromosome 3 linked to the *a* locus. More transpositions were detected in the region 2 to 12 map units distal to *a* and 4 to 30 map units proximal to the *a* locus.

McClintock (1962) has also reported transposition of *Spm* from the *a* locus to linked sites. She observed 33% of the transposed *Spms* were linked to the donor site. Nelson and Klein (1984) obtained a high rate *bz-mutable* alleles when *Spm* was located at the *c* locus on the same chromosome.

Saedler and Nevers (1985) have suggested a cut and paste mechanism for plant transposable elements. The same is likely true for *En*, i.e., *En* transposes by excision from the original site followed by reinsertion at a new location.

2.7. Transposition of *Ac*

The transposition of *Ac* (= *Mp*), is the best characterized transposition mechanism of all the plant transposable elements (Van Schaik and Brink, 1959; Greenblatt and Brink, 1962, 1963; Greenblatt, 1966, 1968, 1974, 1984; Chen et al., 1987).

Transposition studies of *Ac* were studied at the *P-vv* allele of the *P* locus, a gene that controls anthocyanin pigmentation of the pericarp tissue. The *P-vv* allele contains

the *Ac* element inserted in the *P* gene and its expression is blocked as long as the *Ac* remains inserted. Excision of *Ac* from *P-vv* often restores its wild type expression. Because excision only occurs in some cells, the pericarp of a plant carrying *P-vv* is variegated: red stripes (sectors) in a background of non pigmented pericarp. The typical pattern is termed medium variegation.

Occasional early excision events cause ear sectors in which several adjacent kernels have red pericarp. In 26% to 90% of the cases the red sectors have adjacent light variegated sectors. Light variegation shows fewer and later red sectors in the pericarp and is conditioned by the presence of an extra *Ac* element in the genome due to the negative dosage effect of *Ac*. The extra *Ac* arise due to transposition of *Ac* from *P-vv* (Brink and Nilan, 1952). The juxtaposed red and light variegated sectors are named twin sectors.

The kernels from the light variegated co-twin sectors always carry the transposed *Ac* (tr-*Ac*). However, one third of the red co-twin sectors do not carry the tr-*Ac*. Such twins have been identified as type II. The other two thirds of the twins show the presence of tr-*Ac* in the red sector. They have been named type I twins. In case of the type I twins, the tr-*Ac* of the red co-twin is allelic to the tr-*Ac* of the light variegated co-twin (Greenblatt and Brink, 1962). When the tr-*Ac*s of the type I twins are linked to the *P* locus

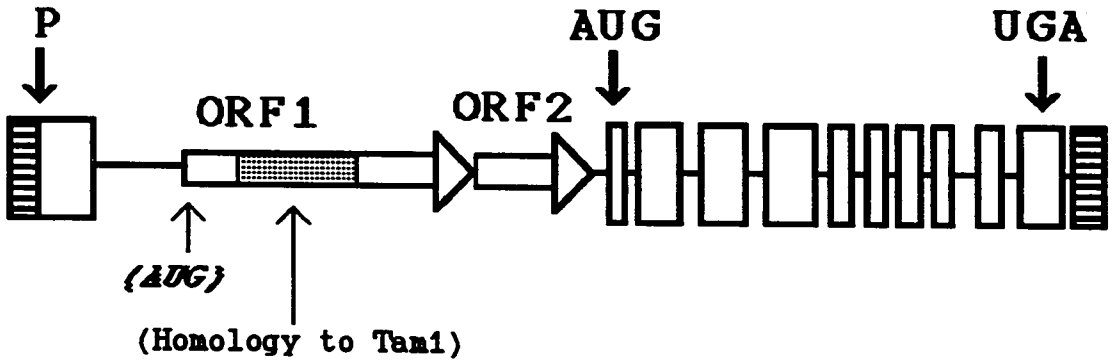
they always map at the same location. This result suggested that the origin of the tr-*Ac* in both members of the co-twin is due to a single transposition event.

These observations have been used to postulate that transposition of *Ac* takes place during chromosome replication (Greenblatt and Brink, 1962; Greenblatt, 1966, 1968, 1974, 1984). Excision of *Ac* occurs from *P-vv* in one of the chromatids after this chromosome segment has replicated. Reinsertion of the *Ac* occurs in a chromosome segment that has not yet replicated. Completion of chromosome replication and mitotic segregation would then result in a transposed *Ac* located at identical chromosome positions in both the resulting sister chromatids. One of the sister cells after mitosis would receive the tr-*Ac* and the original *P-vv* allele. The other cell would receive the tr-*Ac* and the *P* revertant allele from which *Ac* has already excised. If this cell lineage contributes to pericarp formation, the result will be a twin sector of type I.

Alternatively, if reinsertion occurs to a chromosome segment that has already replicated, only one of the chromatids would receive the tr-*Ac*, as opposed to both the chromatids in the previous case. After mitosis, the chromatid may co-segregate with the chromatid carrying the unchanged *P-vv* allele. This event would result in red sectors lacking the tr-*Ac* and would explain the type II twins.

Figure 2.1 The gene structure of *En*

The 8287 bp long *En* element cloned from the *wx-844* allele has eleven exons (open boxes) for the major gene product tnpA. Transcription of this 2.5 kb major transcript is initiated from a weak promotor (P) present at the left end of the element. The first exon is untranslated. The start codon (AUG) for *tnpA* is at the beginning of the second exon and translation up to the end of the eleventh exon produces a 68 kd DNA binding protein shown to represent the *S* function of *En*. The unusually long first intron contains two open reading frames (ORF1 and ORF2). The ORF1 has a homology to the *Tam1* element as shown. The transposase, believed to be *tnpB* (now termed *tnpD*), expresses the two ORFs in a 6 kb minor transcript and uses the first start codon (*AUG*) at the beginning of ORF1.


P
AUG
UGA
ORF1
ORF2
(ΔAUG)
(Homology to Tam1)

3. MATERIALS AND METHODS

3.1. Gene Symbols and Descriptions

Allele	Description
A	Dominant allele of the *A* locus (O'Reilly et al., 1985); necessary for anthocyanin pigment formation; codes for a NADPH dependent dihydroquercetin reductase (Coe and Neuffer, 1977).
a	Recessive allele of *A*; colorless when homozygous.
a-m(Au)	An *En* containing autonomously mutable allele of *A*; coarse sectored to colored aleurone (Peterson, 1961).
a-m1 6078	Recessive allele of *A*; colorless to light pale aleurone in the absence of *En* and coarse sectored in the presence of *En* (Reddy and Peterson, 1984).
a-m1 5719	Recessive allele of *A*; pale to dark pale in the absence of *En*; spotted with pale sectors in its presence (McClintock, 1954; Schwarz-Sommer et al., 1985b).
a-m(r), *a-m(r) 102*	Colorless in the absence of *En*; heavily spotted when *En* present (Peterson, 1961; Cuypers et al., 1988).
a-m2 8004,	Colorless in the absence of *En*; In the presence of *En*, spotted with pale background. Occasionally colorless sectors in the pale background are seen; colorless areas bordered by

	darkly pigmented rings are also a typical feature of this allele in the presence of *En* (McClintock, 1967; Reddy and Peterson, 1985; Schwarz-Sommer et al., 1987).
a-m2 4412, *a-m2 3456*	Colorless without *En*; spotted with various degrees of pale background with *En* (Reddy and Peterson, 1985; Schwarz-Sommer et al., 1987).
A2	Dominant allele of the *A2* locus; necessary for anthocyanin pigmentation (Menssen et al., 1990).
a2	Recessive allele of the above; colorless phenotype when homozygous.
a2-m1 ("A2" state or "state II")	Colored in the absence of *En*; sectored in its presence (McClintock, 1957, 1958; Menssen et al., 1990).
a2-m(r)	Colorless without *En*; spotted with *En*.
C	Dominant allele of the *C* locus; necessary for anthocyanin pigmentation in aleurone tissue (Paz-Ares et al., 1986).
c	Recessive allele of the above; colorless aleurone when homozygous.
c-m(r)	Colorless without *En*; spotted in its presence (Peterson, 1970).
C2	Dominant allele of the *C2* locus (Wienand et al., 1986); necessary for anthocyanin pigmentation; codes for chalcone synthase (Dooner, 1983).

c2	Recessive allele of the above; colorless when homozygous.
c2-m2	Pale colored without *En*; heavily sectored in its presence (McClintock, 1964; Wienand et al., 1986).
Wx	Dominant allele of the *Wx* locus (Shure et al., 1983; Klösgen et al., 1986); Wx starch stains blue to dark purple with I+KI; codes for starch granule bound, UDP-glucose starch glycosyl transferase (Echt and Schwarz, 1981).
wx	Recessive allele of the above; wx starch stains yellowish to reddish brown with I+KI.
wx-m8	Waxy phenotype in the absence of *En* and wx-mutable in the presence of *En* (McClintock, 1961a; Schwarz-Sommer et al., 1984).

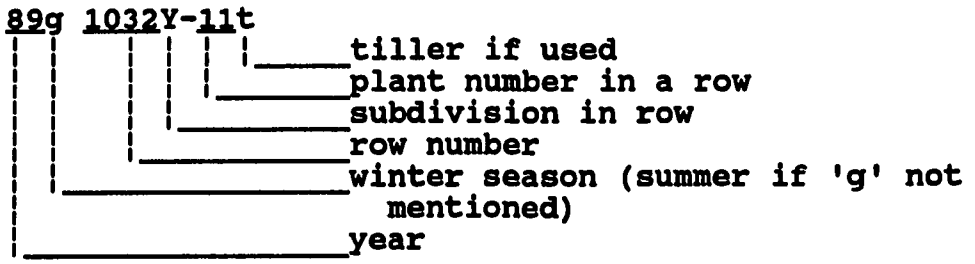
3.2. Abbreviations

Abbreviation	Explanation
a-->A (read as: small a to large A)	spots or sectors in a colorless background and the mutability is at the *A* locus
c-->C (read as: small c to large C)	spots or sectors in a colorless background and the mutability is at the *C* locus.
c2-->C2 (read as: small c2 to large C2)	spots or sectors in a colorless bacground and the mutabilty is at the *C2* locus.

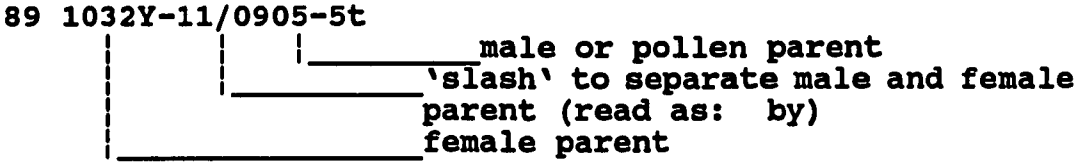
co (read as: coarse)	coarse: refers to a spotting or sectoring pattern showing larger (earlier) and more frequent spots or sectors.
fi (read as: fine)	fine: refers to a spotting or sectoring pattern showing
wx (read as: small waxy)	waxy: Non-staining endosperm by I2+KI
Wx (read as: large waxy)	Non-waxy or starchy: Blue or purple staining endosperm
wx-m	waxy mutable: Sectors of Wx in a background of wx
wx-mutable	waxy mutable: Same as above
wx-->Wx (read as small waxy to large waxy)	waxy mutable
wx-m co or wx-mutable co or wx-->Wx co	waxy mutable coarse: frequent and early or large Wx sectors in a background of wx. Sometimes looks like wx sectors in a background of Wx.
wx-m fi or wx-mutable fi or wx-->Wx fi	waxy mutable fine: relatively small number and sizes of Wx sectors in a background of wx

3.3. Representation of Crosses

3.3.1 Identification of plant in a cross

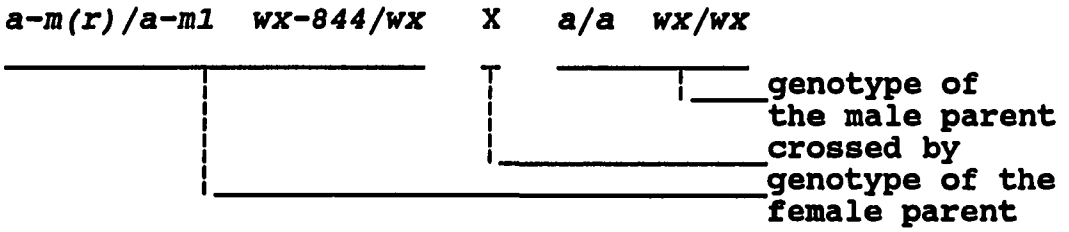


3.3.2 Representation of parents in a cross (with plant numbers)

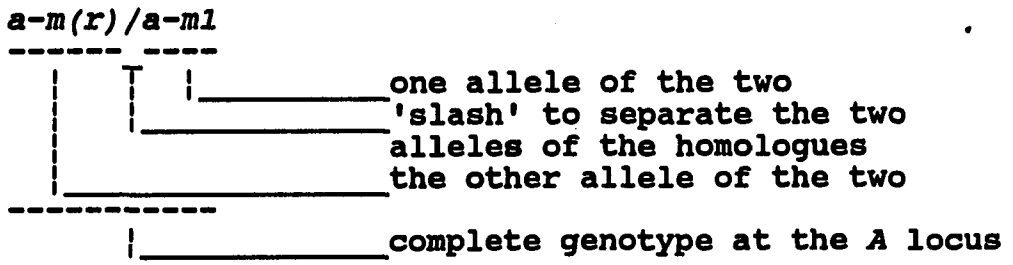


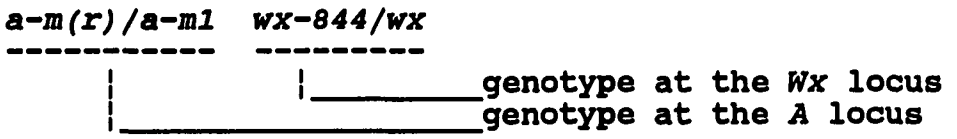
The cross is read as: eighty-nine ten thirty-two Y dash eleven by O nine O five dash five T or eighty-nine O nine O five dash five T on ten thirty-two Y dash eleven.

3.3.3 Representation of parents in a cross (with genotypes)



3.4. Representation of Genotypes





3.5. Response to *En* Functions by Reporter Alleles

Reporter allele	Response to the *En* functions	Description
a-m1 5719	*S, M*	Suppression of pale color is a measure of *S* function; the frequency of spots is a measure of *M* function (Schwarz-Sommer, et al., 1985b).
a-m1 6078	*M*	Large, colored sectors are due to *M* action (Schwarz-Sommer et al., 1985b).
a-m(r)	*M*	Frequency of spots measure the strength of *M* function (Cuypers et al., 1988).

a-m2 8004 *a-m2 4412* *a-m2 3456*	*coex, M*	The background pale coloration reflects coexpression of *A* gene and spots reflect *M* function (Schwarz-Sommer et al., 1987).
a2-m1 ("A2" state)	*S*	Suppression of dark color reflects the *S* function; appearance of colored sectors shows the loss of *S* function (McClintock, 1958; Menssen et al., 1990).
a2-m(r)	*M*	Spots are caused by *M* action.
c-m(r)	*M*	Spots are caused by *M* action.
c2-m2	*S, M*	Disappearance of pale background color is caused by *S* action; the sectors ellicit *M* action (Wienand et al., 1986).
wx-m8	*M*	Appearance of Wx sectors in wx background reflects *M* action (Schwarz-Sommer et al., 1984).

3.6 Spotting Patterns

A variety of aleurone spotting patterns appear with the *En* related mutable alleles. The pattern is described according to the frequency and size of spots. The frequency spots can vary

from a very few to a large number of spots densely packed. They are graded in a scale from 1 to 10. One represents the lower end of the scale and ten represents the higher densely packed spots (Figure 3.1).

The size of spots is described in a scale 'a' to 'e' in an increasing order of size. The lower grade, 'a' represents spot size of a few cells and the upper grade, 'e' represents lage sectors of pigmented regions (Figure 3.1).

The frequency of spots is an indication of number of excision events during the development of the endosperm and the size of spots reflect the timing of the excision event. Early excision causes larger size of spots or sectors and late excision causes small spots.

The terms coarse and fine are also used to describe the spotting pattern. Coarse refers to large number of early sectors and fine refers to fewer and smaller spots. 'Coarse sectored' is also used to describe the coarse spotting pattern.

3.7. Waxy Mutability

3.7.1. Staining

Waxy staining of the endosperm was done by scraping the kernel surface to expose the endosperm tissue. The endospermal tissue contains starch that responds to staining by I2+KI. A diluted solution of I2+KI is applied to the exposed surface.

The cells containing the amylose starch stains dark blue to purple. The cells lacking the amylose starch takes a lighter brown stain. The light brown color is washed off by applying hot water to the surface. Hot water treatment thus, increases the clarity of the staining. In a wx-mutable kernel, groups of cells (sectors) producing amylose starch stain dark while, the cells in the background do not take the dark staining. Hot water washing makes the background clear and amylose starch producing sectors or Wx sectors clearly stand out in a clear background. Scraping of a small area on the kernel surface is adequate to identify the waxy mutability. But, scraping through the middle of the kernel reveals the sectors of Wx very clearly. In general the middle or center of the kernel does not stain very well because of the non-intactness of the cellular structure in the middle.

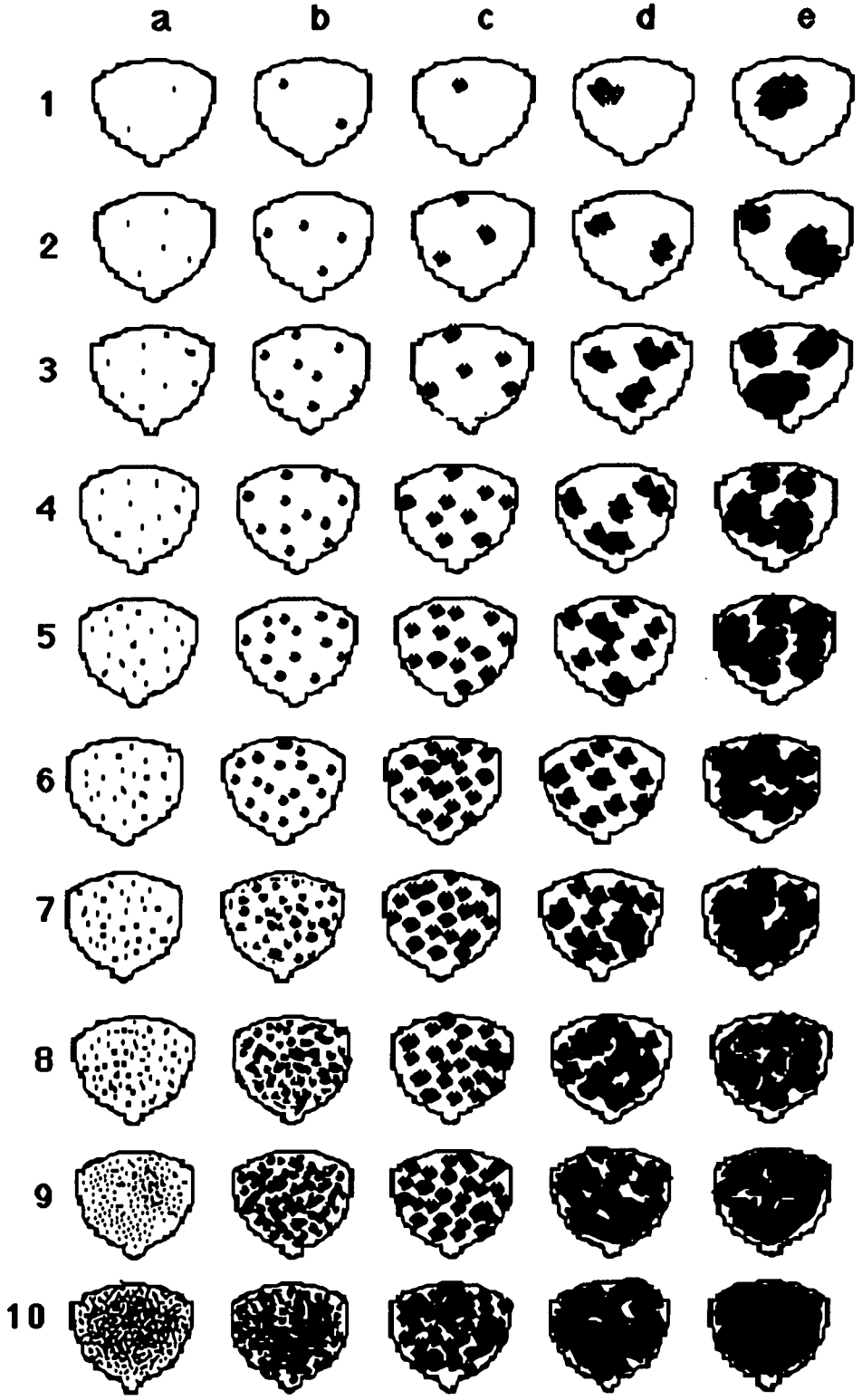
3.7.2. Waxy mutability pattern

Size and number of Wx staining sectors in the endosperm are considered to decide the pattern of wx-mutability. A pattern consisting of a large number of early (large size) sectors is described as a coarse pattern of wx-mutability. In contrast a fine pattern of wx-mutability consists of a fewer number of late (small size) Wx sectors.

A 'low' wx-mutability pattern is also used to describe a fewer number of wx-->Wx sectors.

Figure 3.1. Scale of aleurone spotting pattern

The vertical scale represents an increase in frequency of spots in a scale of 1 to 10. The horizontal scale represents an increase in size of spots in a scale of a to e.


a
b
c
d
e
1
2
3
4
5
6
7
8
9
10

4. RESULTS

4.1. The Repression Effect of the *a-m(r) 102* Allele

4.1.1. Segregation of two waxy mutable patterns

In the original experimentation, the *wx-844* allele was present in combination with two reporter alleles viz., *a-m(r) 102* and *a-m1 5719A*. The genotype was *a-m(r) Sh/a-m1 sh wx-844/wx*.

The following cross was made in an attempt to include the *wx-844* allele individually with each of the *I* elements, i.e., *I-102* at the *a-m(r) 102* allele and *I-5719* at the *a-m1 5719* allele.

a-m(r) Sh wx-844 \/ *a wx* Cross - 1
a-m1 sh wx /\ *a wx*

Among the progeny two quite distinct types of wx-mutability appeared. One type of wx-mutability was a very coarse expression with large Wx sectors occurring early in the development and giving the appearance of a full Wx type with sectors of wx inside them (Figure 4.1 B). On the same ear a second type was present with a finer reduced form of wx-mutability occurring later in the development (Figure 4.1A).

4.1.1.1. Association of wx-mutability with the *I* element present From an examination of the progeny of this cross, it could readily be seen that there is a one to one correspondence between the wx-mutable pattern in the endosperm and the spotting pattern in the aleurone. The *a-m1 5719* allele shows a medium spotting pattern with a number of large pale colored sectors. This is always associated with the coarse wx-mutability (Figure 4.1B). The *a-m(r) 102* allele shows a very late and high (frequency) aleurone spotting pattern and in this cross this allele is always associated with the fine (late) wx-mutability (Figure 4.1A). This segregation pattern is shown in Table 1.

4.1.1.2. Reappearance of coarse wx-mutability from fine selections when separated from the *a-m(r)* allele The high spotted fine wx-mutable kernels were selfed and crossed to medium spotted coarse wx-m kernels in order to separate the *wx-844* allele from *a-m(r)*. The result among the selfed progeny is shown in Table 2a. Coarse wx-mutability reappeared among the progeny and only in the colorless class. The colorless class lacks the *a-m(r)* allele. Fine wx-mutability was retained among the high spotted kernels. This class contains the *a-m(r)* allele which causes the spots.

In the sib cross shown in Table 2b coarse wx-mutability also reappeared but only among the medium spotted and color-

less class, i.e., in the absence of the *a-m(r)* allele. Fine wx-mutability appeared only among the high spotted kernels, i.e, in the presence of the *a-m(r)* allele.

As a control, the medium spotted coarse wx-m kernels were also selfed. Only coarse wx-m appeared among the progeny confirming that coarse wx-m is the basic or unmodified pattern of the *wx-844* allele.

4.1.1.3. Reappearance of fine wx-mutability from coarse wx-mutable selections when *a-m(r)* is reintroduced The colorless wx kernels from cross 1 and shown in Table 1 are of the genotype *a-m(r)/a wx/wx*. This genotype was used to introduce *a-m(r) 102* by crossing to medium spotted coarse wx-m kernels lacking the *a-m(r)* allele. The result in Table 3 shows fine wx-mutability reappeared among the progeny. Only the high spotted kernels showed fine wx-mutability confirming the correlation between presence of *a-m(r)* and fine wx-mutability.

As a control the medium spotted coarse wx-m selections were also crossed to colored or pale wx selections. Only coarse wx-m appeared in this cross. This result shows that another factor besides *a-m(r)* is not responsible for modifying the coarse wx-m to a fine pattern. The presence of such a factor would have been detected among 15 such crosses made.

4.1.2. Transposition of *I-102*

The *a-m(r) 102* allele contains an *I* insertion in the *A* gene (Schwarz-Sommer et al., 1987). This *I* element has been named *I-102*.

Transposition of this *I* element to a position independent of *A* has been obtained and is shown here. A very low spotted kernel(2b) appeared as an exception among the progeny of the cross, *a-m1/a-m1 wx/wx* X *a-m(r)/a-m1 wx-844/wx-844*. The expected genotype of all round kernels from this cross should be *a-m(r)/a-m1;* but, when back crossed to the *a/a wx/wx* tester, the ear segregated for only 1/4 spotted kernels (Flow diag. 1). The *wx-844* allele was segregating as expected, i.e., 1/2 and among them, coarse and fine were segregating. There was an excess of colorless kernels. The spotted wx-m fine class was showing a decidedly lower spotting pattern (Figure 4.5) when compared to the normal spotting pattern of *a-m1* as seen among the spotted wx-m coarse class. The appearance of wx-m fine suggests that the repressing factor for wx-mutability is still present. Excess of the colorless class suggests that a change has occurred at the *a* locus. Absence of the *a-m(r)* spotting pattern in the cross suggests that the change has occurred at the *a-m(r)* allele. The excess of colorless and presence of *En* (wx-mutability) in this class suggests that the allele is no longer responsive to *En*. This allele is termed *a-nr* for non-

responsiveness. Among the spotted kernels 1/2 showed fine wx-mutability and the other half coarse wx-mutability. This segregation pattern shows that the factor for repression of wx-mutability is independent of the *A* locus. This was confirmed by back crossing the spotted low wx-m fine kernels. The resulting progeny and expected ratios are shown in Flow diag. 1.

These observations suggest that the *I-102* element inserted in the *A* locus has transposed to a new site independent of the *A* locus and is still capable of modifying excision of *En* at the *wx-844* allele to a fine pattern. The recovered *I-102* element independent of the *a-m(r)* allele was used in subsequent experiments. The colorless wx-m fine kernels are of the genotype, *a/a* and also carry the *I-102* element. The *a/a* genotype was used to study the repression of excision at other mutable alleles at the *A* locus viz., *a-m(Au)*, *a-m1 6078*, etc. without the complication arising from *a-m(r)* mutability.

4.1.3. Suppression of *a-m(Au)* phenotype by *I-102*

The *a-m(Au)* allele shows colorless sectors in a colored background (Figure 4.2A) and when used as a female parent shows almost a fully colored phenotype. The term coarse sectored (Figure 4.2B) is used to describe this phenotype.

Availability of *a/a* the genotype with *I-102* made it possible to study the repression effect of *I-102* on *a-m(Au)*.

The *wx-844* allele was used to monitor the presence of *I-102* in this experiment. A control cross, *a-m(Au)/a-m(Au) wx/wx* X *a/a wx-844/wx* was made to verify that the presence of another *En* does not affect the *a-m(Au)* phenotype. In this cross, the coarse sectored pattern of *a-m(Au)* persisted in the wx-mutable coarse kernels, i.e., in the presence of *wx-844* and was indistinguishable from the coarse sectored kernels in the wx class. This observation proves that the *a-m(Au)* phenotype remains unaffected in the presence of another *En*, in this case *wx-844*.

The *I-102* element was combined with *a-m(Au)* in the cross, *a-m(Au)/a wx/wx* X *a/a wx-844/wx-844 I-102/-*. Only wx-mutable kernels from this cross were analyzed. Kernels that lack the *I-102* show the wx-m coarse phenotype and kernels carrying the *I-102* show the wx-m fine phenotype. Among the wx-m coarse kernels two aleurone phenotypic classes were observed, coarse sectored and colorless. The colorless phenotype is due to *a/a*. Among the wx-m fine kernels a new phenotype appeared in addition to that of colorless. It was spotted 6-7b (Figure 4.2 A and B). The presence of this spotting pattern among the wx-m fine kernels indicates that *a-m(Au)*'s coarse sectored pattern might have been repressed to this 7b pattern. Lack of this phenotype among the wx-m

coarse class also agrees with this assumption (in a control cross of similar nature but lacking the *I-102* element the 7b spotting pattern did not appear as shown in Figure 4.2 A). If this assumption is true, the original phenotype of *a-m(Au)*, i.e., coarse sectored should reappear from the spotted 7b kernels when separated from *I-102*. To do this, the spotted 7b wx-m fi kernels were crossed by the tester *a/a wx/wx*. Among the progeny, two phenotypes appeared, coarse sectored and spotted 7b, confirming these expectations. In a control cross with the same tester, the coarse sectored wx-m coarse kernels showed only the original phenotype of *a-m(Au)*. These results suggest that the high mutability of *a-m(Au)* is also repressed in the presence of the *I-102* to a finer pattern. The result is outlined in Flow diag. 2.

4.1.4. Suppression of *a-m1 6078* mutability pattern by *I-102*

The *a-m1 6078* allele shows a colorless to light pale phenotype in the absence of *En* and a coarse sectored phenotype in the presence of *En* (Figure 4.3 A and B, bottom ears). The phenotype is very similar to that shown by the *a-m(Au)* allele.

An *a/a* genotype containing the *I-102* element and the *wx-844* allele as the source of *En* was crossed to the *a-m1 6078* allele homozygous for *wx*. The cross was, *a-m1 6078/a-m1 6078 wx/wx* X *a/a wx-844/wx I-102/-*. In this cross the pres-

ence of the *I-102* was monitored with the mutability pattern at the *wx* locus. The wx-m coarse phenotype indicates the absence of *I-102* and wx-m fine indicates the presence of it. Among the progeny of the cross, a new spotted phenotype appeared. This new pattern was 6b and appeared only among the wx-m fi kernels, i.e., in the presence of *I-102*. In a control cross of a similar nature but lacking the *I-102* element, no fine spotted kernels appeared (Figure 4.3 A, bottom). The absence of this spotted 6b phenotype among the wx-m coarse kernels and its presence only among the wx-m fine kernels suggested that the new phenotype could be due to the repression of the *a-m1 6078* phenotype by the *I-102* element. If true, this new phenotype should revert back to its original coarse sectored type when separated from the *I-102* element. The spotted 6b wx-m fine kernels were test crossed to *a/a wx/wx*. The coarse sectored wx-m coarse kernels were also test-crossed in the same way as a control. The control segregated back only coarsely sectored aleurone phenotype (Figure 4.3 B, bottom ear), whereas the other selections gave both phenotypes, i.e., spotted 6b wx-m fine and coarse sectored wx-m coarse (Figure 4.3 B, top ear). These results suggest that the mutability pattern of the *a-m1 6078* allele is also repressed to a lower and late excision pattern in the presence of the *I-102* element.

4.1.5. Suppression of *a-m1 5719* spotting pattern in the presence of *I-102*

This result was derived from the crosses initially made to establish the transposition of the *I-102* in Section 4.1.2. The cross was *a-m1/a-m1 wx/wx* X *a-m(r)/a-m1 wx-844/wx-844*. A spotted low(2b) exceptional kernel was found in this cross and when test crossed by *a/a wx/wx*, revealed to carry a transposed *I-102* element. The transposition occurred from the *a-m(r) 102* allele and as a result gave rise to an *a-nr* allele at the *a* locus. The genotype of the exceptional kernel was confirmed to be *a-m1/a-nr w-844/wx*. The test cross of this exceptional low spotted kernel to *a/a wx/wx* showed two types of spotted phenotypes. The low spotting was found among the kernels with fine wx-mutability pattern. The original phenotype of *a-m1 5719* in the presence of *En* reappeared among the coarse wx-mutable kernels. A further round of crosses of the spotted low wx-m fine class to *a/a wx/wx* giving with the same result confirmed that the low spotted-(2b) phenotype is due to the repression by *I-102*. The difference between the original and modified phenotypes of *a-m1* is shown in Figure 4.4 A, B, and C.

An important observation is noted here with the spotted low phenotype. They have completely colorless background with excision spots scattered on the aleurone. The frequency of spotting is reduced. But there is no effect on the back-

ground coloration (Figures 4.4 and 4.5). The *a-m1 5719* allele gives a pale color in the absence of *En*. This color is suppressed in the presence of *En*. The colorless background suggests that the *I-102* element has no effect on the *S* function of *En*; but affects only the *M* function that is seen as a reduction in the excision of the *I* element.

4.1.6. Effect of *I-102* on the co-expressing *a-m2 4412* allele

The *a/a wx-844/wx I-102/-* genotype was crossed to the *a-m2 4412/a-m2 4412 wx/wx* genotype to see the effect of *I-102* on the co-expression ability of *En* (Figure 4.6, top). Co-expression is represented by pale coloration and spots. A similar cross was made as a control in the absence of *I-102* and is shown in Figure 4.6, bottom ear. The *a-m2* allele in the presence of *En* gives a pale background with the excision spots superimposed on it. The pale background shows variability in its expression (Figure 4.6, bottom ear).

The following observations are noted from the progeny of this cross. The frequency of excision, i.e., the spotting pattern of *a-m2 4412* is reduced among the wx-m fine kernels but the pale background persists (Figure 4.7 B). The pale background appears darker among these kernels than in the sib kernels showing wx-mutability coarse pattern (Figure 4.7 A). The darker background may be due to the dull looking texture of wx-m fine kernels compared to the shiny appearance of the

wx-m coarse kernels. That the pale background color persists implies that the co-expression ability of *En* is not affected by the presence of *I-102*.

4.1.7. Suppression of *c-m(r)* spotting pattern in the presence of *I-102*

The *c-m(r)* allele gives a 7c-d spotting pattern in the presence of *En* (Figure 4.8 A). The *a-m(r) 102* allele was combined with *c-m(r)* in two steps as outlined in Flow diag. 4. Two kinds of F1s were made using *a-m(r)/a-m(r) wx-844/wx-844* one with the *c-m(r)* and the other with the *c* allele. The two F1s were crossed with each other to expose the *c-m(r)* allele. In this cross, 3 distinct types of spotting pattern appeared. One was clearly *c-->C* pattern shown by the *c-m(r)* allele in the presence of *En* (Figure 4.8 A), another was *a-->A* pattern that is shown by the *a-m(r)* allele in the presence of *En* (Figure 4.1A). A third type was very low spotting pattern of irregularly shaped small spots (Figure 4.8 B). It was assumed to be the suppressed form of the *c-m(r)* spotting pattern. The three different types were each crossed to a *c/c* tester, the types showing *c-->C* were also crossed to the *a/a* tester. The outcome of these crosses are shown in Flow diagram 4.

The original pattern of *c-m(r)* spotting is described here as *c-->C* coarse (Figure 4.8 A), the lower spotting

pattern is described here as *c-->C* fine (Figure 4.8 B) and the *a-m(r)* spotting pattern is described here as *a-->A* (Figure 4.1A). The *c-->C* coarse types segregated back only the same pattern when crossed to *c/c* tester and only colored and/or mottled when crossed to *a/a* tester, thus indicating the absence of *a-m(r)* in the genotype. The mottled phenotype is due to the *r* allele segregating in the line.

The *c-->C* fine type in a testcross to *c/c* segregated both *c-->C* coarse and *c-->C* fine types. Expectedly, also, they showed *a-->A* kernels on the *a/a* test cross indicating the presence of the *a-m(r)* allele. The *a-->A* selections when crossed to *c/c* gave back *c-->C* fine type only.

This result shows a complete correlation between the presence of *a-m(r)* and appearance of *c-->C* fine spotting pattern. When *c-->C* coarse is selected the appropriate test cross shows the absence of *a-m(r)*. When the *c-->C* fine is selected, the testcross detects the presence of *a-m(r)*. When *a-m(r)* homozygous is selected, *c-->C* fine is the only *c-m(r)* spotting pattern present. This correlation proves that the *c-m(r)* coarse pattern is suppressed to a reduced fine pattern (Figure 4.8D) in the presence of *a-m(r) 102*.

4.1.8. Suppression of *c2-m2* spotting pattern in the presence of *a-m(r) 102*

A strategy similar to section 4.1.7 for *c-m(r)* was also adopted for the *c2-m2* allele. This is shown in Flow diag. 5. The two types of F1s, one bringing the *c2-m2* and the other bringing the *c2* allele were crossed together to expose the *c2-m2* allele in order to visualize its spotting pattern. Three different spotting patterns appeared in this cross. A very coarse pattern that resembled a mottled type which is typical when *c2-m2* is present with *En* (Figure 4.10 A, right). Another, lower pattern as shown in Figure 4.10 A, left. The third pattern was an *a-m(r)* spotting pattern (Figure 4.1A). Each of these types were crossed to *c2/c2* tester and the *c2-m2* types were also crossed to *a/a* tester. The result is summarized in Flow diag. 5.

The *c2-->C2* coarse (Figure 4.10A, right) types on a backcross to *c2/c2* segregated only the coarse type and the pale type (*c2-m2* phenotype in the absence of *En*). In test-crosses to *a/a*, only pale and colored appeared indicating the absence of *a-m(r)*. The *c2-->C2* fine kernels (Figure 4.10A, left) when crossed to *c2/c2* segregated both coarse and fine back (Figure 4.9 A and B). As expected, the fine type also showed *a-->A* spotting in testcrosses to *a/a* indicating the presence of *a-m(r)*. Some of this group showed only *c2-->C2* fine kernels, in that, no coarse appeared. Their correspond-

ing *a/a* testcross showed only *a-->A* and colorless indicating homozygosity for the *a-m(r)* allele. These kernels were never separated from the *a-m(r)* allele to give back the coarse pattern of *c2-m2*. The *a-m(r)* spotted selections when crossed to *c2/c2* gave only *c2-->C2* fine kernels.

These results also show a complete correlation between the presence of *a-m(r)* and the presence of the *c2-->C2* fine phenotype. The presence of *c2-->C2* fine is always associated with the presence of *a-m(r)* and the absence of *c2-->C2* fine is always associated with the lack of *a-m(r)* in the line. These results indicate that the *a-m(r) 102* allele represses the coarse pattern of *c2-m2* to a lower mutability pattern (Figure 4.10 A and B).

4.1.9. The reducer, *En-malt* of *c-m5 5292* is also detected as an *a-m(r)* allele

The *c-m5 5292* allele shows a coarse spotting pattern. In the presence of the factor *En-malt* the pattern is suppressed to a finer type. The nature of this phenomenon is similar to that found with the suppression of *wx-844* coarse mutability to a fine wx-mutability. Crosses were made between plants from kernels showing the fine mutability at *c-m* and plants carrying the *wx-844* allele without any suppressor *I-102*. Fortuitously, as would be seen later, the *wx-844* genotype was also homozygous for *a*. A control cross was also

made between *a/a wx-844/wx* and *c-m* coarse genotypes. In the control cross the resulting ears showed all colored kernels, as is expected (Figure 4.11B). The *c-m* fine with *wx-844* cross gave three classes of phenotypes viz. colored (the only expected), spotted and colorless (Figure 4.11 A). The result is shown in Table 4a.

The spotted kernels had the same pattern as that seen with the *a-m(r)* allele in the presence of *En*. The presence of spotted and colorless kernels was not expected in a cross of such a nature. Since the *wx-844* parent was also homozygous for *a*, it was argued that an *En* responding allele was also present in the *c-m* fine parent. The phenotype of the responding allele resembles that of the *a-m(r)* allele. This indicates that the suppression of *c-m* coarse may be due to *a-m(r)* itself and was never detected since it remained in heterozygous condition with the *A* allele. The cross with the *a/a* allele exposed the *a-m(r)* allele.

If the above argument is true, the segregation pattern of the three classes should be 4 Cl : 3 sp : 1 cl. This is expected with 2 *En*s, one being at *wx-844* and the other at *c-m*. The two ears shown in Table 4a fits this expectation. The proof that this *a-m(r)* suppresses is derived from the wx-m fine kernels that are detected only in the spotted class and not in the colored class. The segregation pattern also fits the expectations.

These results indicate that the *En-malt* present in the *c-m5 5292* line is an *a-m(r)* having the same kind of suppressing capability of *a-m(r) 102* that was discovered as a suppressor of coarse wx-mutability.

A similar cross was made with the sib kernels of the genotype *c/c wx/wx* with or without *En-malt* and the result also corroborates with the above conclusion. The source of the seeds were from the cross, : *c-m5 5292/c wx/wx En-malt/-* X *c/c wx/wx*. The colorless kernels selected were of the genotype, *c/c wx/wx* and half of them should carry *En-malt* and the other half should lack *En-malt*. Therefore, in a cross of these colorless kernels to *a/a wx-844/wx*, half should expose the *a-m(r)* allele and the other half should lack the *a-m(r)* allele. This would be true if *a-m(r)* is equivalent to *En-malt* in these lines. Eighteen such crosses were made. Nine of them showed the segregation pattern shown in Table 4b and the other nine showed only colored kernels among the progeny as shown in Table 4c. These results are in agreement with the expectations that the unexposed or hidden *a-m(r)* is equivalent to *En-malt*.

4.1.10. Dosage effect of *a-m(r)*

While working with the *a-m(r)* and *wx-844* crosses it was observed that the fine mutability varies in its appearance in various crosses. The distinct feature was the number of *wx*--

>Wx sectors. A systematic attempt was made to vary the dosage of the *a-m(r)* allele and the *wx-844* allele in the kernels to see whether the dosage is the cause of such variability in the wx-mutability pattern among the wx-mutable fine kernels. The maize endosperm being triploid, the dosage of each of the alleles can be varied from 1 to 3. When the allele is used as a male, it will contribute 1 dose; when used as female, it will contribute 2 doses; and when used as both male and female the progeny will carry 3 doses of the corresponding allele. This basis has been used in constructing the different dose combinations shown in Table 5.

Representative kernels from each of the 9 dose combinations are shown in Figure 4.13 (A-F). The observation illustrates that keeping the dose constant, one dose of *a-m(r)* is least effective in suppressing. When dosage of *a-m(r)* is increased, the frequency of *wx-->Wx* sectors is reduced. That is, 3 doses of *a-m(r)* has more suppressing capacity than 1 dose of *a-m(r)*. When the *a-m(r)* dose is kept constant and the *wx-844* dosage is increased, the frequency of wx-mutability is also increased. The relationship appears linear.

4.1.11. Other *I* elements tested for suppression of wx-mutability at the *wx-844* allele

The following mutable alleles containing *I* insertions were crossed to the *wx-844* allele to observe if they will suppress the coarse mutability of *wx-844*. *a-m1 5719*, *a-m2 8004*, *a2-m(r)*, *c2-m2*, *c-m(r)* and *a2-m1* were tested for this purpose. In all the crosses the original coarse wx-mutability of *wx-844* was maintained indicating that none of the above *I* elements are capable of eliciting the suppressor effect of *a-m(r) 102*.

Table 1. Segregation of two waxy mutable patterns and their association with the spotting pattern at the *A1* locus corresponding to the reporter allele present

Cross:	*a-m(r)102* / *a-m1 5719*	*Sh2* / *sh2*	*wx-844* / *wx*	X	*a* / *a*	*Sh2* / *Sh2*	*wx* / *wx*

Genotypes:	*a-m(r)* / *a* / *wx-844* / *wx*	*a-m1* / *a* / *wx-844* / *wx*	*a-m(r)* / *a* / *wx* / *wx*	*a-m1* / *a* / *wx* / *wx*
Phenotypes: wx-mutability spotting	(A) fine high	(B) coarse medium	(C) waxy c1	(D) waxy C1
860131/0201-4	59	65	65	57
860132/0201-6	75	84	68	50 @
860132/0201-7	86	71	77	67
860131/0203T	49	73	52	69
860215Z-3/0131	42	40	48	50
	(A)	(B)	(C)	(D)

Crosses with selections from (A), (B), (C) and (D):

A (x) and A X D: Tables 2a and 2b
Reappearance of coarse wx-mutability from fine wx-mutable selections when separated from the *a-m(r)* allele.

B X C: Table 3
Reconstitution of fine wx-mutability from coarse wx-mutable selections when *a-m(r)* is reintroduced.

B (x): Only coarse wx-mutable appears among selfed progeny: proves coarse is the basic wx-mutable pattern (data not shown).

B X D: Only coarse wx-mutable appears among this sib cross progeny: rules out the possibility of another factor besides *a-m(r) 102* being involved in repression (data not shown).

X^2 for 1:1:1:1 is not significant at 5% for all entries except @.

Table 2a. Reappearance of coarse wx-mutabilty among selfed progeny

Cross:	*a-m(r) 102* / *a*	*wx-844* / *wx* (x)	
Genotypes: @	*a-m(r)* / *a-m(r)/a* / *wx-844* / *wx-844/wx*	*a* / *a* / *wx-844* / *wx-844/wx*	*a-m(r)/a* / *a-m(r)/a* / *wx* / *wx*
Phenotypes: wx-mutabilty spotting	fine high	coarse cl	waxy cl
875340-5	188	60	102
875340-10	178	53	75

X^2 for 9:3:4 is not significant at 5%.
@: '/' indicates one of the two alleles present.

Table 2b. Reappearance of coarse wx-mutabilty among sib cross progeny

Cross:	*a-m(r) 102* / *a*	*Sh2* / *Sh2*	*wx-844* / *wx*	X	*a-m1 5719* / *a*	*sh2* / *Sh2*	*wx* / *wx*
Genotypes: @	*a-m(r)* / *a-m1/a* / *wx-844* / *wx*	*a-m1* / *a* / *wx-844* / *wx*	*a* / *a* / *wx-844* / *wx*		*a-m(r)/a* / *a* / *wx* / *wx*		*a-m(r)/a* / *a-m1* / *wx* / *wx*
Phenotypes: wx-muta-bility	fine	coarse	coarse		waxy		waxy
spot-ting	high	medium	cl		cl		pale
875340-9/ 5341-12	101	49	66		101		96

X^2 for 2:1:1:2:2 is not significant at 5%.
@: '/' indicates one of the two alleles present.

Table 3. Reconstitution of fine wx-mutabilty when *a-m(r)-102* is reintroduced

Cross:	*a-m(r) 102 Sh2 wx* / *a Sh2 wx*	X	*a-m1 5719 sh2 wx-844* / *a Sh2 wx*

Genotypes@	*a-m(r)* / *a-m1/a* / *wx-844* / *wx*	*a-m1* / *a* / *wx-844* / *wx*	*a* / *a* / *wx-844* / *wx*	*a-m(r)/a* / *a* / *wx* / *wx*	*a-m(r)/a* / *a-m1* / *wx* / *wx*
Phenotypes:					
wx-mutabilty	fine	coarse	coarse	waxy	waxy
spotting	high	medium	cl	cl	pale
875344y-4t/5343z-1	68	35	32	71	60
875346y-3/5347x-1	66	35	28	74	77

X^2 for 2:1:1:2:2 is not significant at 5%.
@: '/' indicates one of the two alleles present.

Table 4a. Apppearance of *a-m(r)* in crosses involving *En-malt*

Cross:
c-m/c wx/wx En-malt/- X *a/a wx-844/wx*

		Cl*	sp**	cl***	chi sq (4:3:1)
87 1246-1/1223-4t	1.	256	176	53	1.9
87 1246-6/1223-12t	2.	242	169	42	4.84

*1. 205 kernels showed 1:1 segregation of wx-m co and wx with 3 Wx exceptios.
2.241 kernels showed 1:1 segregation of wx-m co and wx with 1 Wx exception.

**1. 92 kernels showed 1:1 segregation of wx-m fi and wx with 2 Wx exceptions.
2. all kernels showed 1:1 segregation of wx-m fi and wx with 2 Wx exceptions.
***1. 20 tested wx.
2.40 tested wx.

Table 4b. Appearance of *a-m(r)* in crosses involving *En-malt*

Cross: *c/c wx/wx En-malt/-* X *a/a wx-844/wx*				
	Cl*	sp**	cl	chi sq. (2:1:1)
87 1247-7/1224-10t	197	110	97	1.08
87 1248-1/1223-12t	254	143	105	5.83

*Sample checked showed wx-m co and Wx.
**Sample checked showed wx-m fi and Wx.

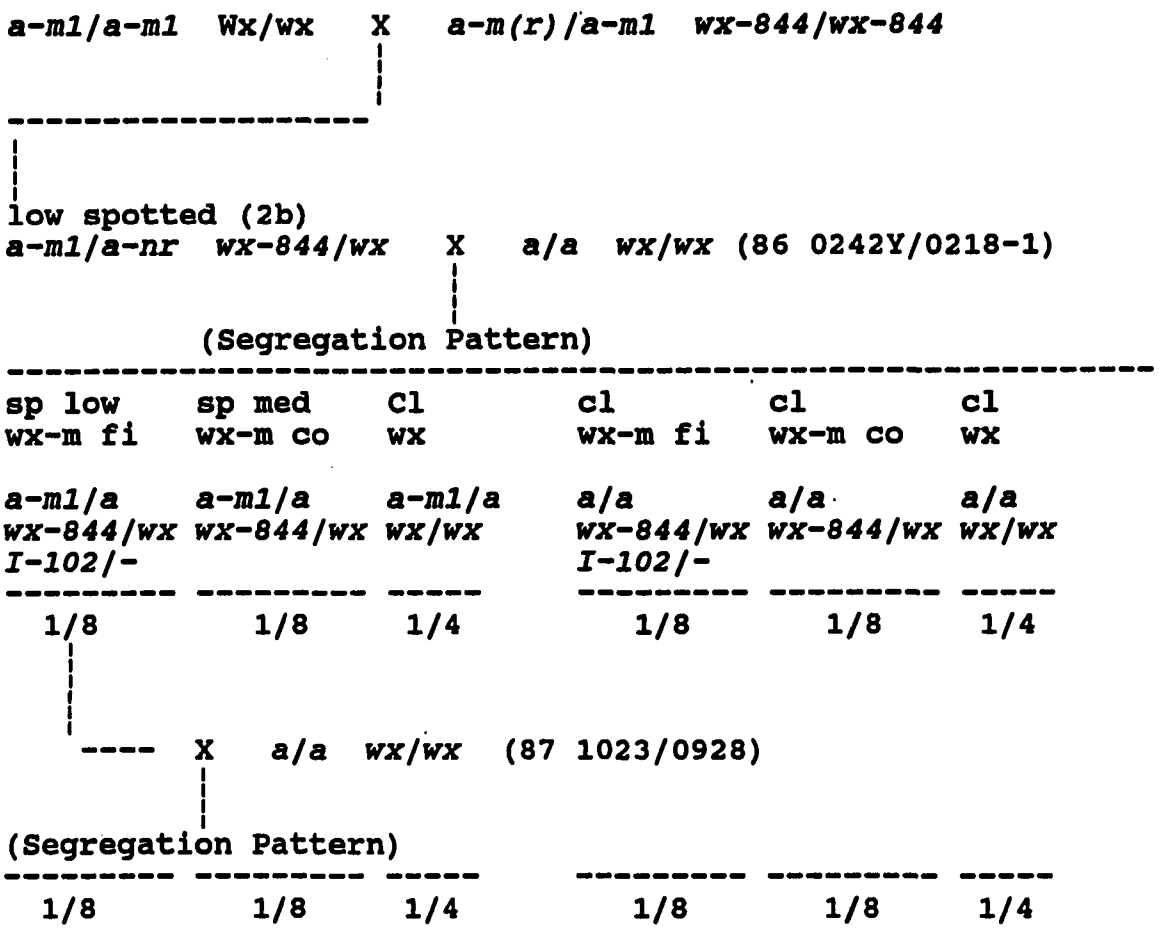
Table 4c. Appearance *a-m(r)* in crosses involving *En-malt*

Cross: Same cross as in 4b.				
	wx-m co	All colored wx	Wx	chi sq. (1:1)
87 1248-6/1223-6t	37	40	13	1.11
87 1248-3/1223-12t	30	36	0	0.545

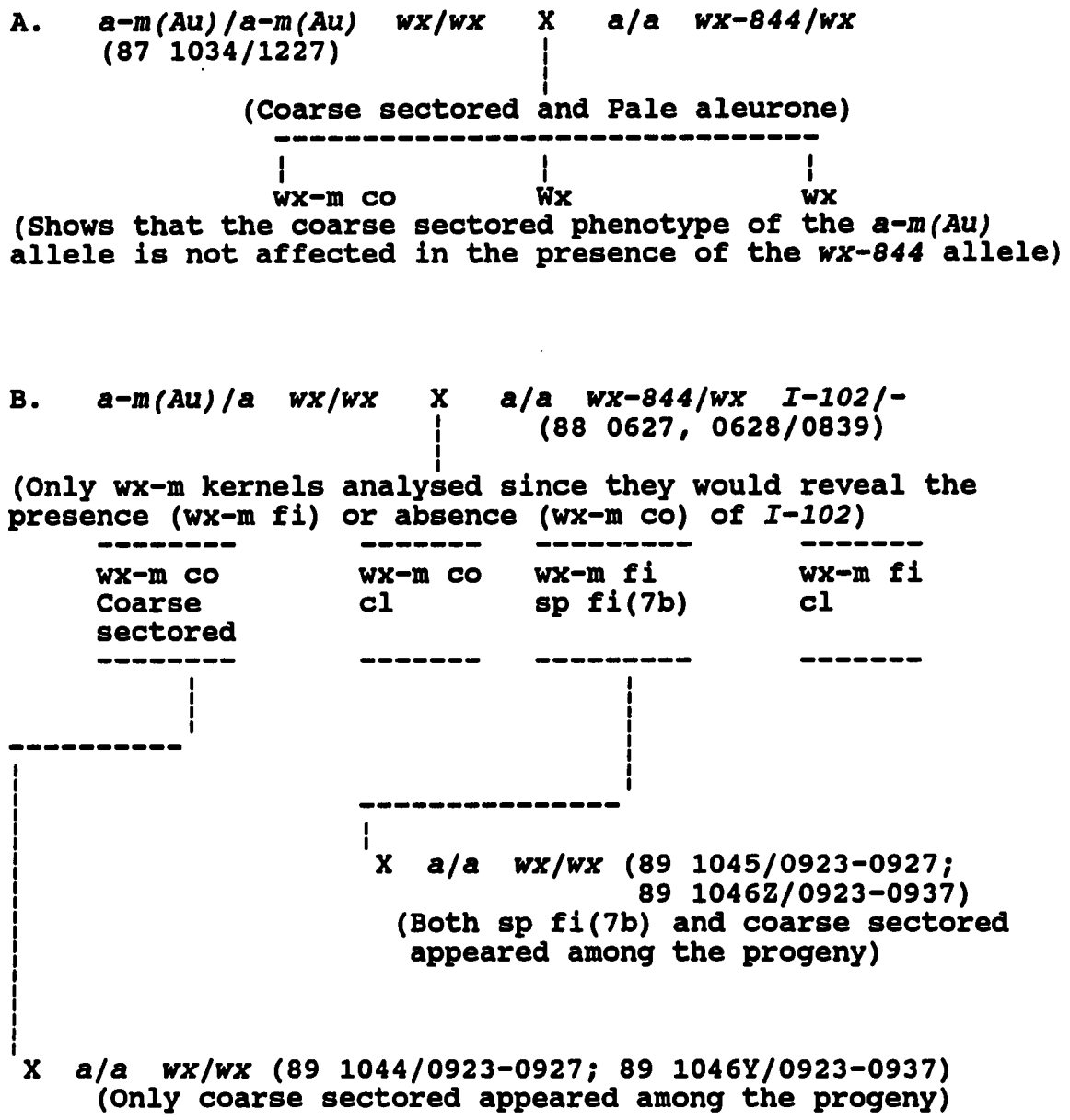
Table 5. Construction of genotypes for *a-m(r) 102* and *wx-844* dosage

Cross	Genotypes	Dose *wx-844*	Dose *a-m(r)*
86 0131/ 0201-4	*a/a wx/wx* X *a-m(r)/a-m1 wx-844/wx*	1	1
87 5344Y-3t/ 5343Z-1	*a-m(r)/a wx/wx* X *a-m1/a wx-844/wx*	1	2
88 0858-10/ 0603-10t	*a-m(r)/a-m(r) wx/wx* X *a-m(r)/a-m(r) wx-844/wx-844*	1	3
88 0634-3/ 0858-1	*a/a wx-844/wx* X *a-m(r)/a-m(r) wx/wx*	2	1
87 5340-9/ 5341-12	*a-m(r)/a wx-844/wx* X *a-m1/a wx/wx*	2	2
88 0603-8t/ 0858-10	*a-m(r)/a-m(r) wx-844/wx-844* X *a-m(r)/a-m(r) wx/wx*	2	3
88 0857-14/ 0602-1t	*a/a wx-844/wx-844* X *a-m(r)/a-m(r) wx/wx*	2	3
88 0602-5/ 0855-4	*a-m(r)/a-m(r) wx-844/wx-844* X *a/a wx-844/wx-844*	3	2
87 1359-7 *selfed*	*a-m(r)/a-m(r) wx-844/wx-844* *selfed*	3	3

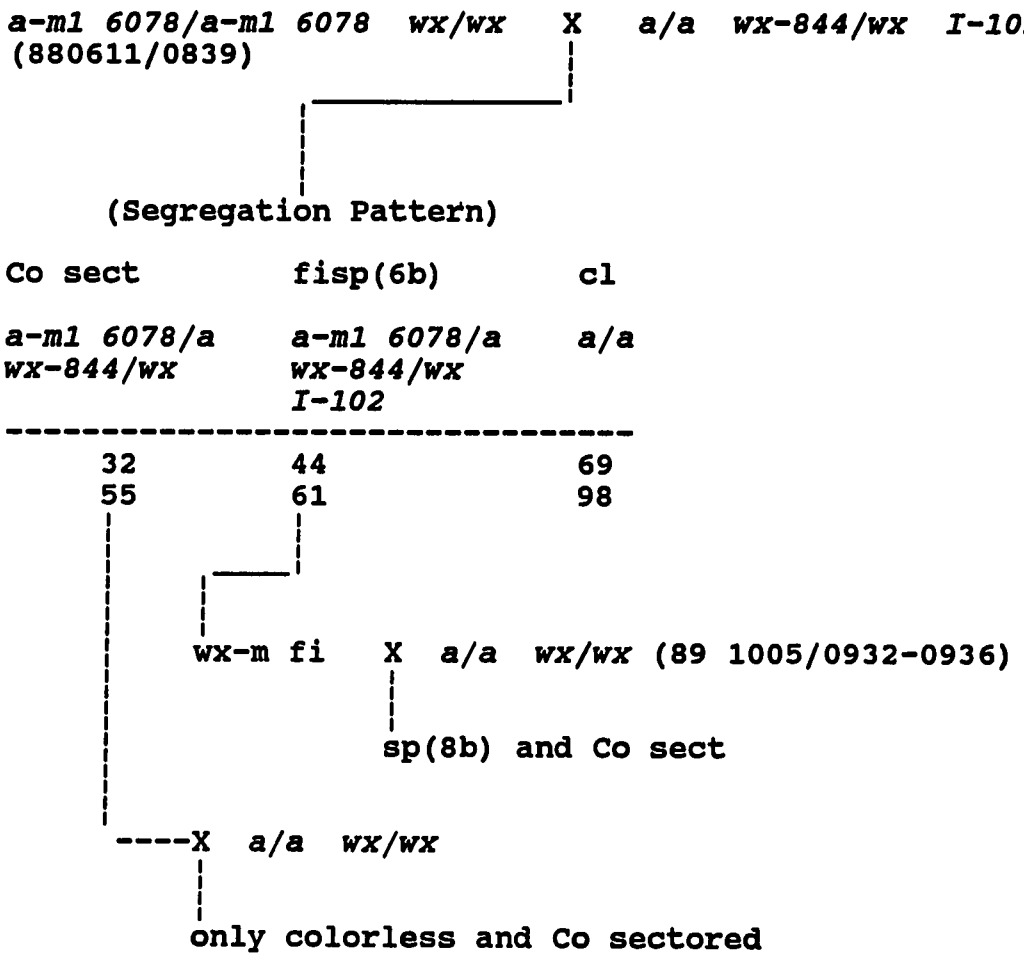
Flow diagram 1. Transposition of *I-102*



Flow diagram 2. Suppression of *a-m(Au)* phenotype by *I-102*



Flow diagram 3. Suppression of *a-m1 6078* phenotype by *I-102*



Flow diagram 4. Suppression of *c-m(r)* phenotype by *a-m(r)* *102*

c-m(r)/c-m(r) *Wx/Wx* X *a-m(r)/a-m(r)* *wx-844/wx-844*
(87 4136/1355-7)

Colored (A)
[88g 196]

c/c *wx/wx* X *a-m(r)/a-m(r)* *wx-844/wx-844*
(87 2958/1356-9)

Colored (B)
[88g 195]

(A) X (B) (88g 196/195-3, 195-11, 195-17[a], 195-17[b])

(Selections)	X *c/c* *wx/wx*	X *a/a* *wx/wx*
--- *c-->C* Co wx-m Co (89 1007Y, 1008Y, 1009Y)	*c-->C* Co only	Colored and or mottled
--- *c-->C* fi wx-m fi (89 1007X, 1008X, 1009X)	*c-->C* Co and fi	*a-->A* appeared
--- *a-->A* wx-m fi (89 1007Z, 1008Z, 1009Z)	only *c-->C* fi appeared	when tested No colored
---Colored	not tested	not tested
---colorless	not tested	not tested

Flow diagram 5. Suppreesion of *c2-m2* phenotype by *a-m(r) 102*

c2/c2 Wx/Wx X *a-m(r)/a-m(r) wx-844/wx-844*
(87 3361/1356-2)

Colored (A)
[88g 197]

c2-m2/c2-m2 or c2 Wx/Wx X *a-m(r)/a-m(r) wx-844/wx-844*
(87 3725/1355-3)

Colored (B)
[88g 198]

(A) X (B) (88g 197-10/198, 88g 198/197-6)

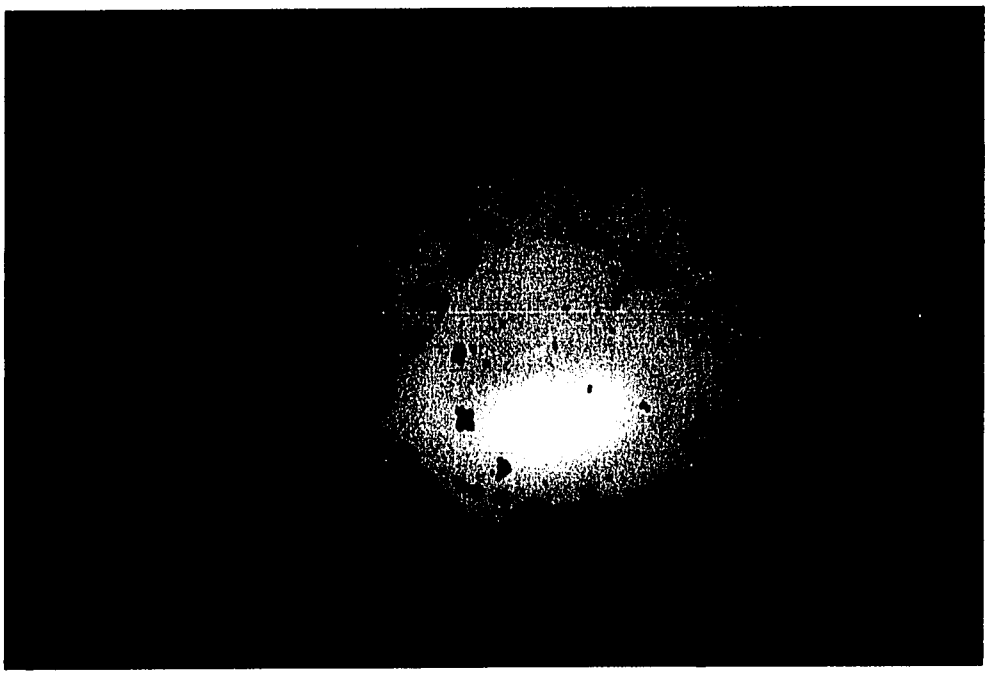
Selections		X *c2/c2*	X *a/a wx/wx*
(89 1010Y, 1011W, 89 1011 Y,Z)	*c2-->C2* Co wx-m Co	*c2-->C2* Co an pale	Colored and pale
(89 1010X, 1011X)	*c2-->C2* fi wx-m fi	i) *c2-->C2* Co and fi ii) *c2-->C2* fi only	i) *a-->A* and colored ii) *a-->A*, no colored
(89 1010Z, 1011Z)	*a-->A* wx-m fi	*c2-->C2* fi only	when tested, no colored appeared
	Colored	not tested	not tested
	colorless	not tested	not tested

Figure 4.1 Two waxy mutable patterns of the *wx-844* allele.

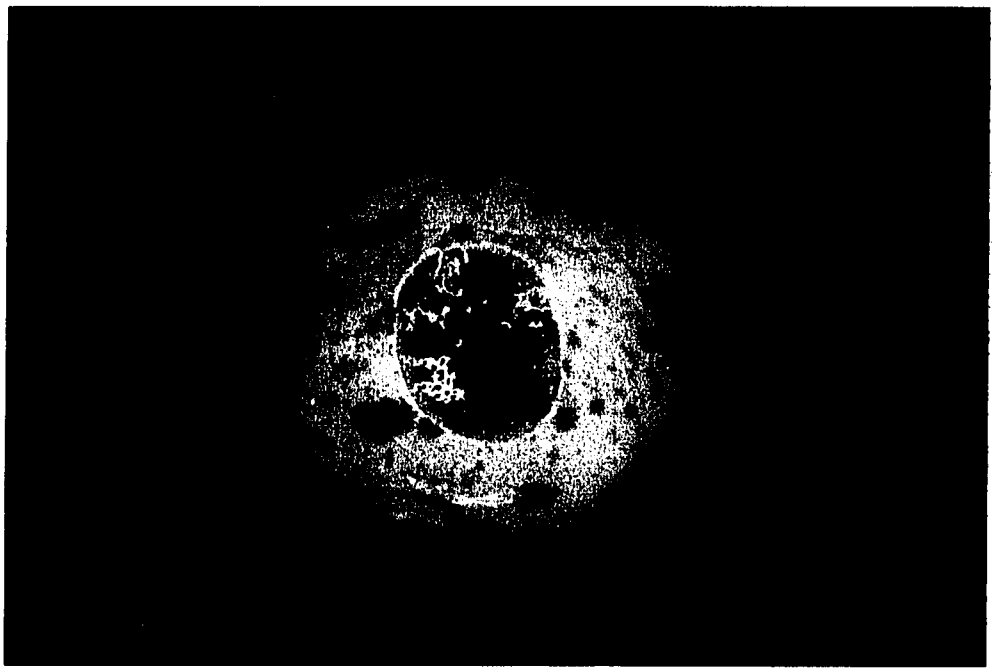
Kernels A and B are derived from the cross:
a/a wx/wx X a-m(r) 102/a-m1 5719 wx-844/wx

A. Fine wx-mutabilty of the *wx-844* allele observed with the genotype *a-m(r)/a wx-844/wx*; i.e., in the presence of the *a-m(r) 102* allele. In this cross, fine wx-mutability always cosegregates with the high spotted aleurone phenotype, a characteristic feature of the *a-m(r) 102* allele.

B: Coarse wx-mutabilty of the *wx-844* allele with the *a-m1 5719* allele; i.e., in the absence of the *a-m(r) 102* allele. The genotype of the kernel is *a-m1 5719/a wx-844/wx*. In this cross, coarse wx-mutability always cosegregates with the medium spotted kernels with pale sectors, a characteristic feature of the *a-m1 5719* allele.



A

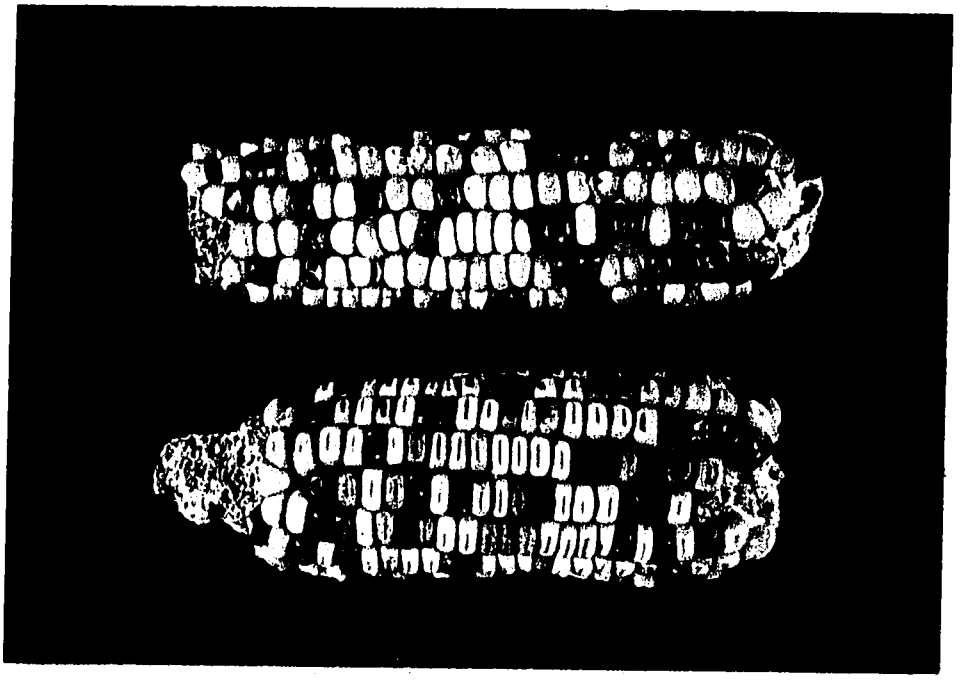


B

Figure 4.2 Suppression of the *a-m(Au)* spotting pattern by *I-102*

A. top: *a-m(Au)/a wx/& X a/a wx-844/wx.*
The only spotting pattern present is that of the unmodified *a-m(Au)* in this cross which is in the absence of *I-102.*
bottom: *a-m(Au)/a wx/& X a/a wx-844/wx I-102/-.*
Two spotting patterns appeared in this cross, the *a-m(Au)* type and the 6-7b type

B. left: *a-m(Au)*'s coarse sectored pattern in the absence of *I-102.* Genotype: *a-m(Au)/a wx-844/wx*
right: suppressed spotting pattern of *a-m(Au)* in the presence of *I-102.* Genotype: *a-m(Au)/a wx-844/wx I-102/-*



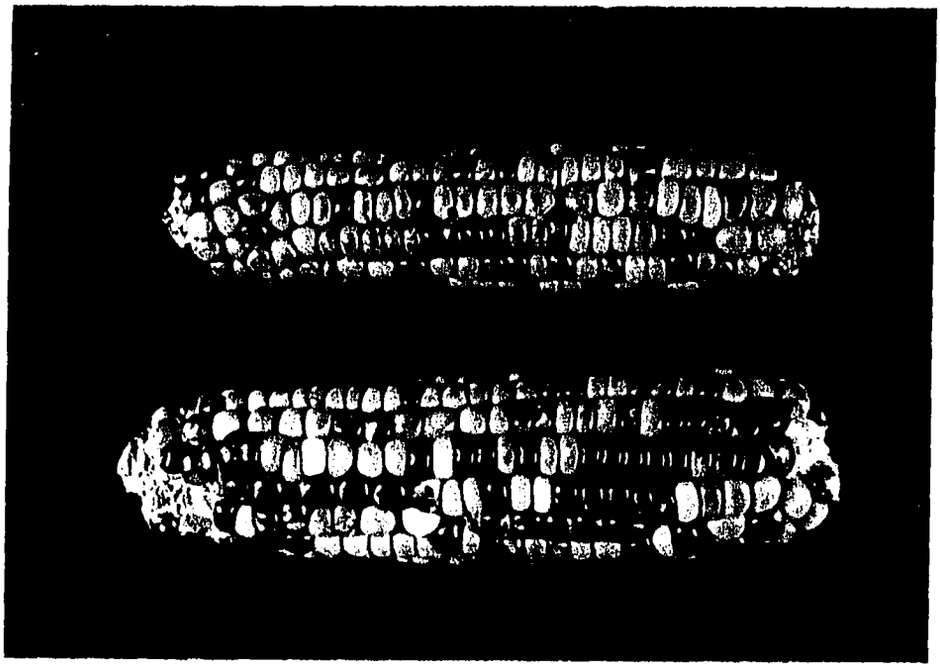
A

B

Figure 4.3 Suppression of the *a-m1 6078* spotting pattern in the presence of *I-102*

A. top: *a-m1 6078/a-m1 6078 wx/wx X a/a wx-844/wx I-102/-*
Two types of spotted kernels appeared in this cross; the original pattern of *a-m1 6078* and the suppressed pattern (6-7b)
bottom: *a-m1 6078/a-m1 6078 wx/wx X a/a wx-844/wx*.
Only the original pattern of *a-m1 6078* appeared

B. top: spotted 6b selections from above (A, top) back crossed to *a/a wx/wx*. Both original and modified spotting pattern appeared
bottom: original *a-m1 6078* phenotype selection from above (A, top) back crossed to *a/a wx/wx*.
Only the original pattern appeared
In all of the above four ears, the spotted 6-7b kernels showed wx-mutable fine phenotype and the kernels with the original pattern of *a-m1 6078* showed wx-mutable coarse or Wx phenotype.



A

B

Figure 4.4 Suppression of the *a-m1 5719* spotting pattern by *I- 102*

A. *a/a wx/wx X a-m1 sh/a-m1 sh wx-844/wx-844 I-102/-*.
Both medium (original pattern of *a-m1*) and low spotting pattern segregating.

B. Original phenotype of *a-m1 5719* in the presence of *En*. Genotype: *a-m1 5719/a wx-844/wx*

C. Suppressed low spotting pattern of *a-m1 5719* in the presence of *I-102*. Genotype: *a-m1 5719/a wx-844/wx I-102/-*.
The kernels in B and C originated from the cross: *a/a wx/wx* X *a-m1 5719/a wx-844/wx I-102/-*.

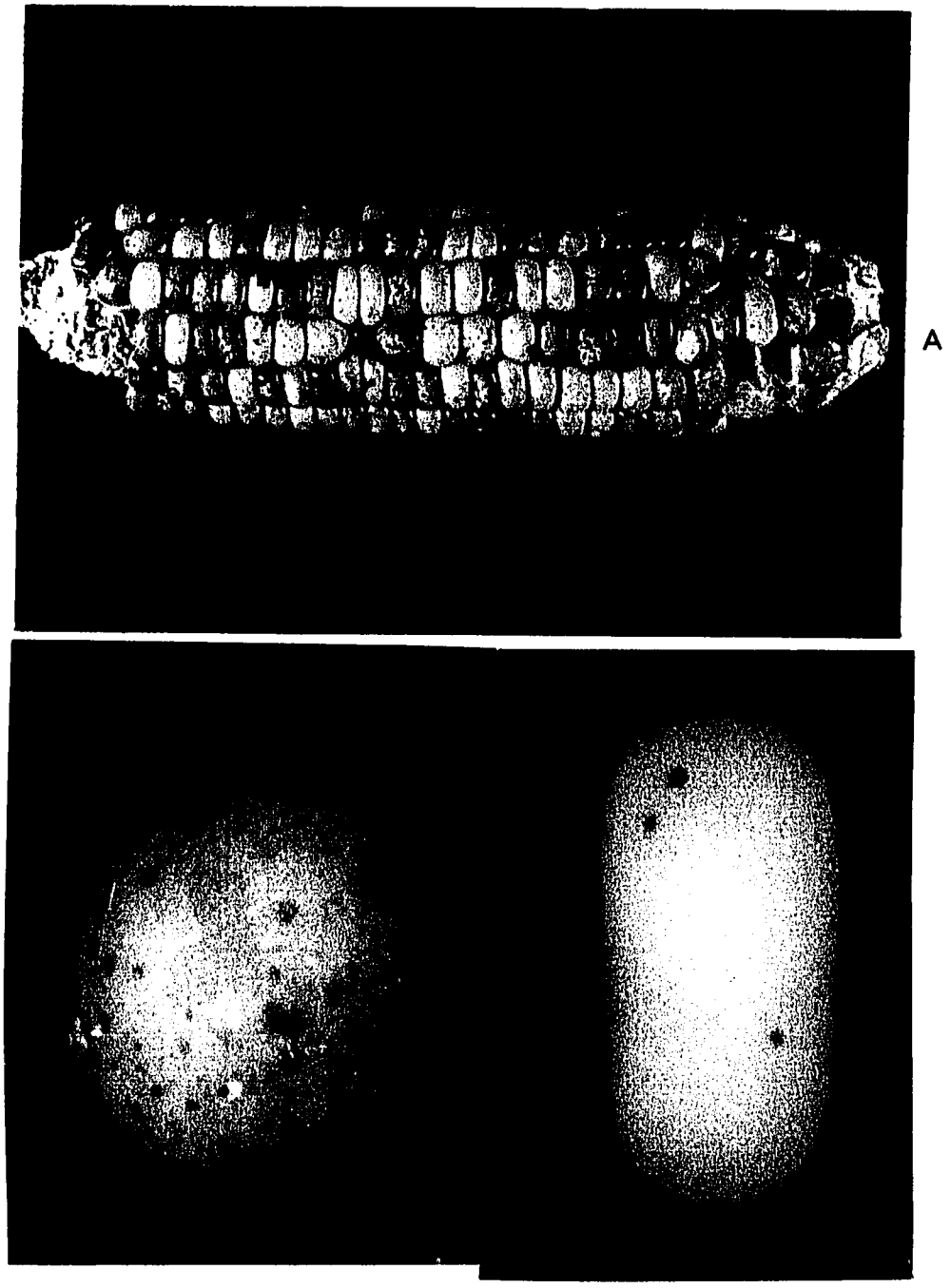

A
B
C

Figure 4.5 *a-m1 5719* phenotype in the presence of *I-102*.

Note the pale background coloration of *a-m1* is not present. Shows *I-102* does not affect the *S* function of *En*. The kernel was obtained from the cross: *a-m1 5719/a wx-844/wx I-102/-* X *a/a wx/wx*. Fine wx-mutabilty in the scraped area confirms the presence of *I-102*

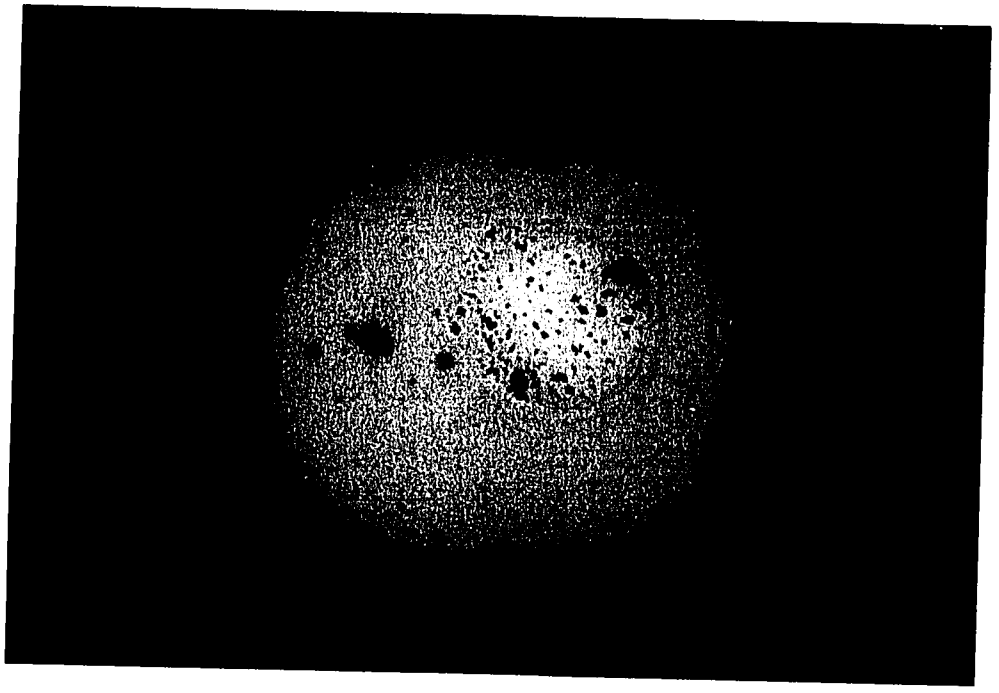


Figure 4.6 Co-expression of *a-m2 4412* allele in the presence of *I-102*

top: *a-m2 4412/a Wx/wx X a/a wx-844/wx I-102/-*. The pale background color persists among the low spotted kernels (a-m2 4412/a wx-844/wx I-102/-). Shows that coexpression is not affected although excision frequency is reduced.

bottom: same cross as top, but in the absence of *I-102*. Note only higher spotting frequency is present. Also shows the range of co-expression of *a-m2 4412* allele in the presence of *wx-844* The range of co-expression is comparable in both the crosses, i.e., coexpression remains unaffected in the presence of I-102

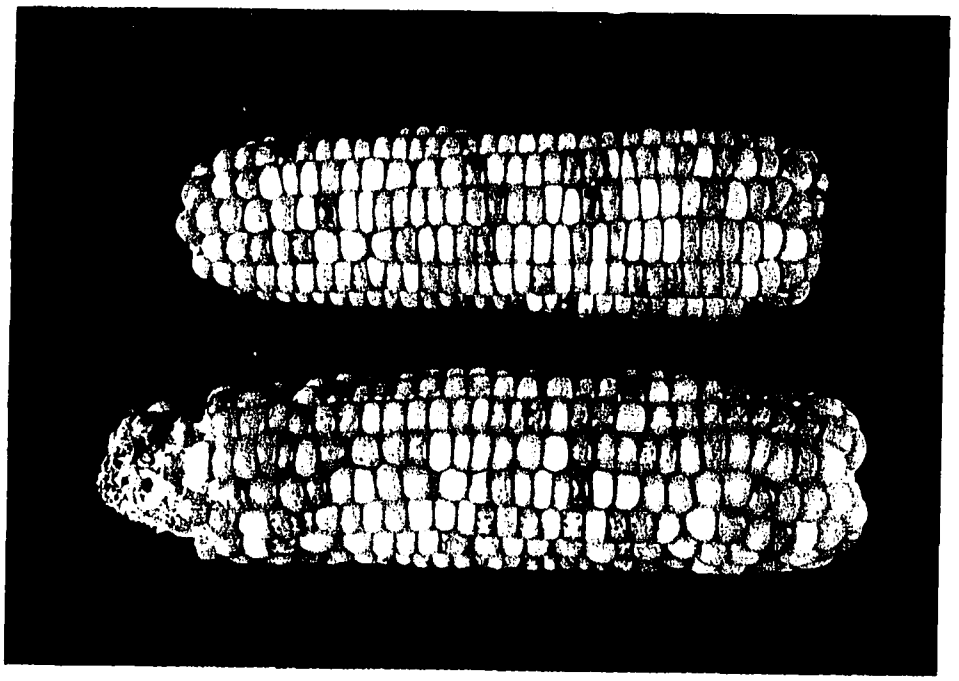
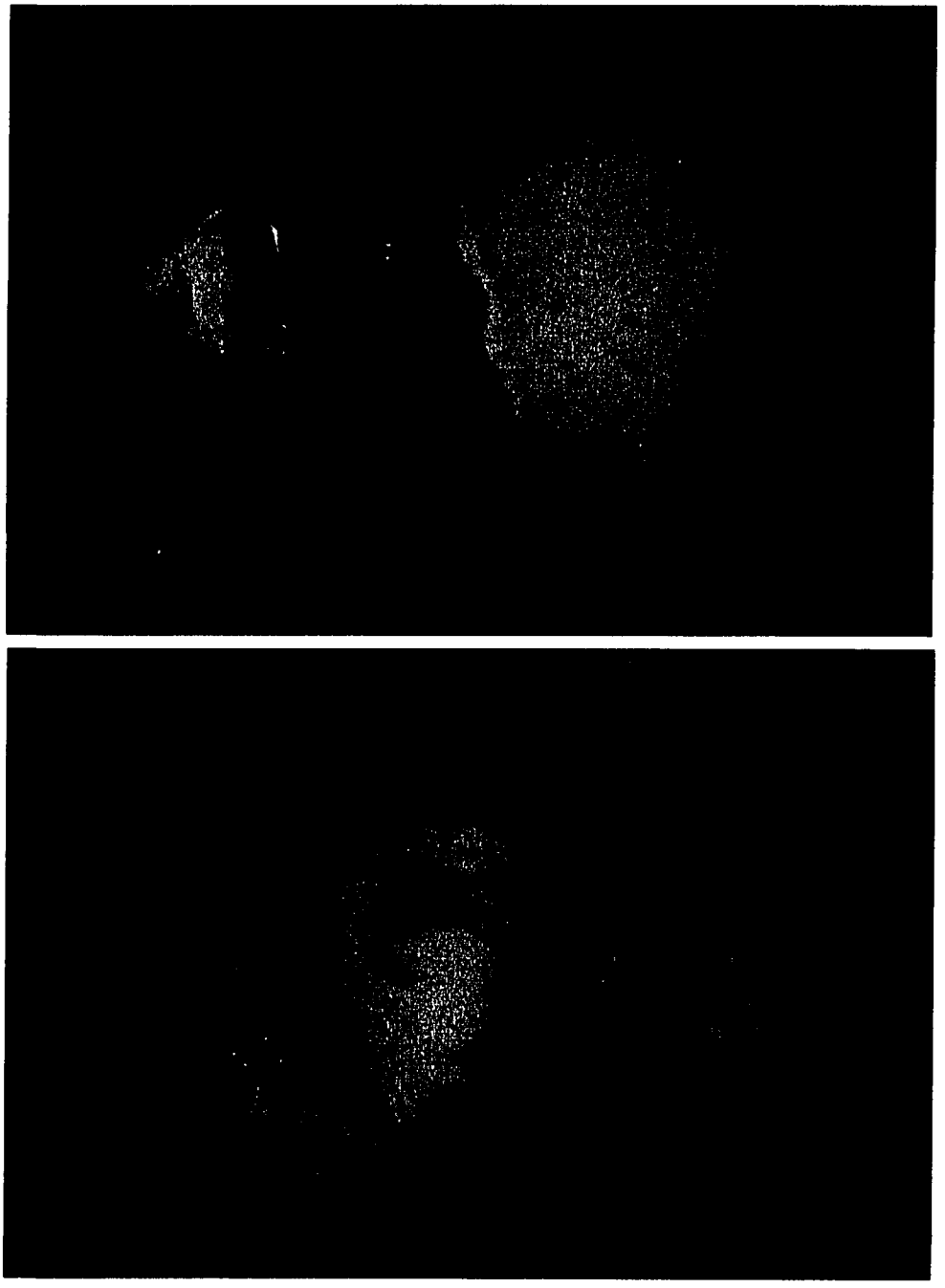


Figure 4.7 Co-expression at *a-m2 4412* allele is not affected by the presence of *I-102*

A. *a-m2 4412* allele with *wx-844*. No *I-102* is present in this genotype (a-m2 4412/a wx-844/wx) as shown by the coarse wx-mutability

B. *a-m2 4412* allele with *wx-844* and *I-102*. The fine wx-mutability indicates the presence of *I-102* (*a-m2 4412/a wx-844/wx I-102/-*). Note the reduced spotting pattern. Also shows the pale co-expression persists



A

B

Figure 4.8 Original and suppressed phenotypes of *c-m(r)*

A. Original phenotype of *c-m(r)* in the presence of En. Genotype: c-m(r)/c-m(r) or c wx-844/wx

B. Suppressed phenotype of *c-m(r)* when a-m(r) 102 is present. Genotype: c-m(r)/c-m(r) or c wx-844/wx a-m(r)/A

C. Original phenotype of *c-m(r)* indicating the absence of *a-m(r)*, since, coarse wx-mutability is present; same genotype as in A.

D. Suppressed phenotype of *c-m(r)* in the presence of *a-m(r)* shown by the presence of fine wx-mutability; same genotype as in B

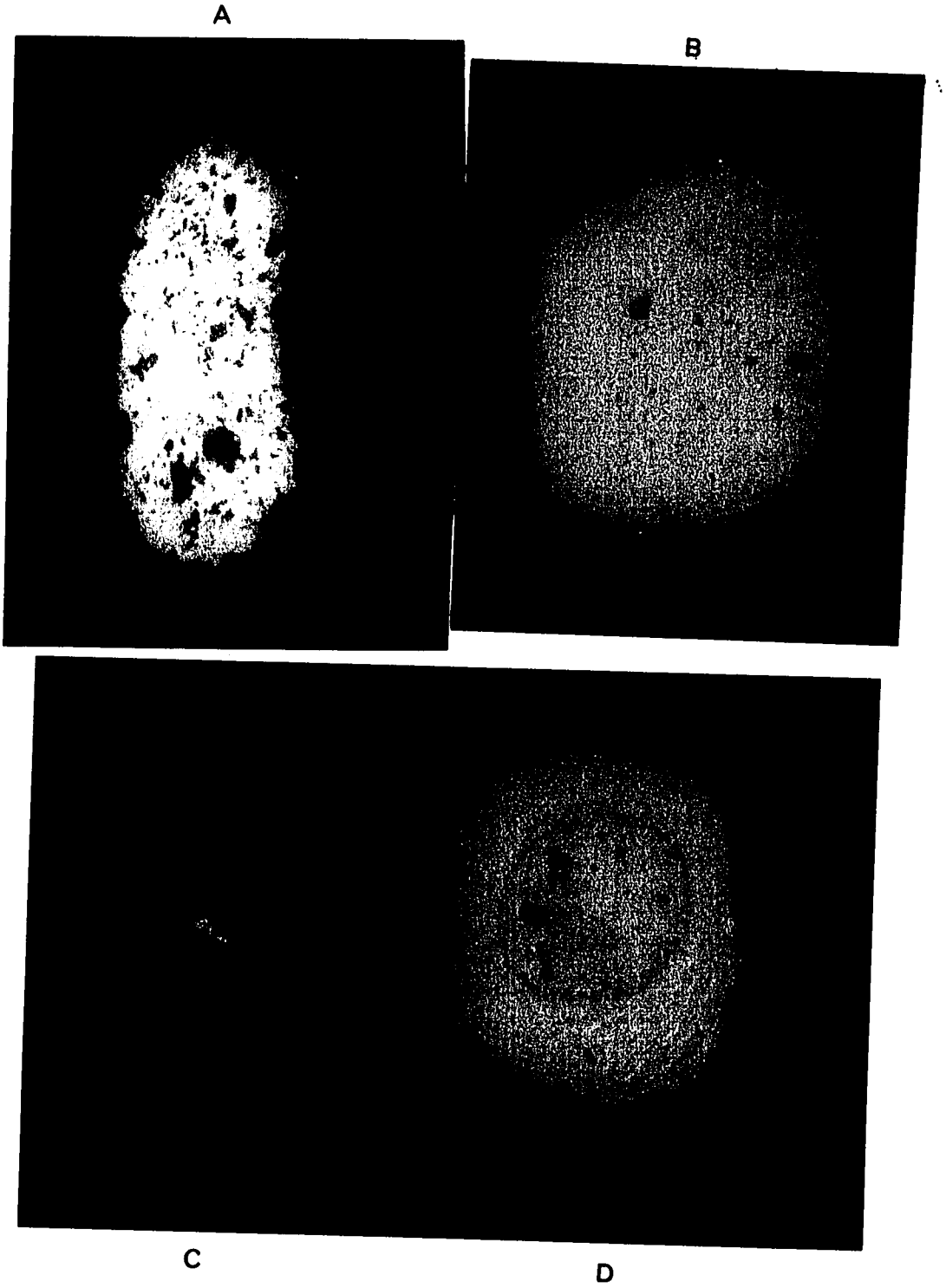
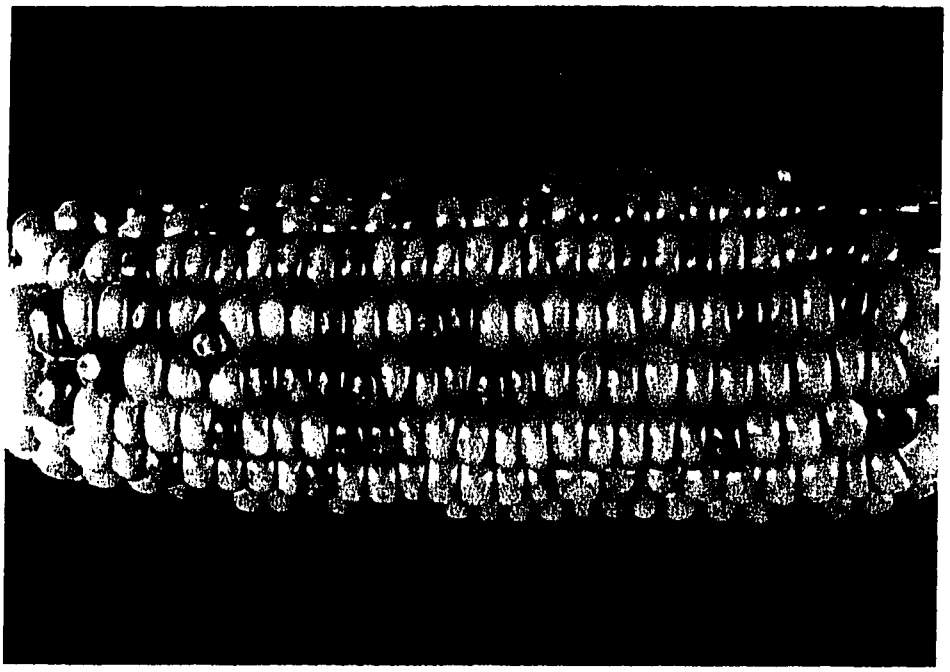

A
B
C
D

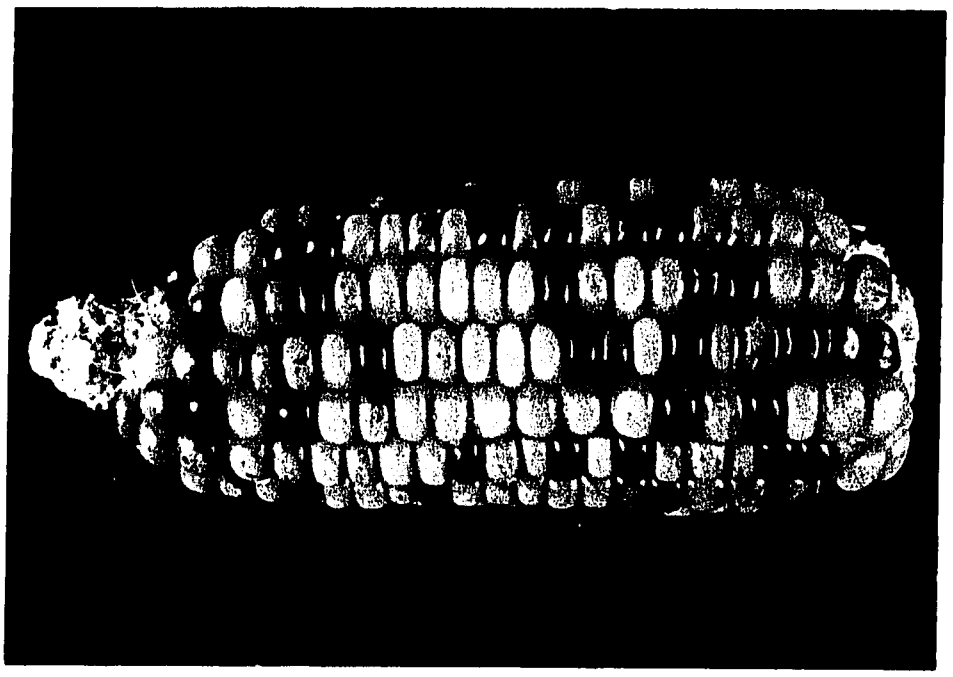
Figure 4.9 Segregation of original and reduced spotting patterns of *c2-m2*

A. Segregation coarse(almost colored with colorless sectors) and fine spotting patterns of *c2-m2*. Both *c2-m2* and *wx-844* originated from the male parent in the cross: *c2/c2 Wx/Wx* X *c2-m2/c2 wx-844/wx-844 or wx a-m(r)/A*.

B. Same as above but the *c2-m2* and *wx-844* originated from the female parent (reciprocal of the cross shown in A). Note the darker and higher spotting than in A



A

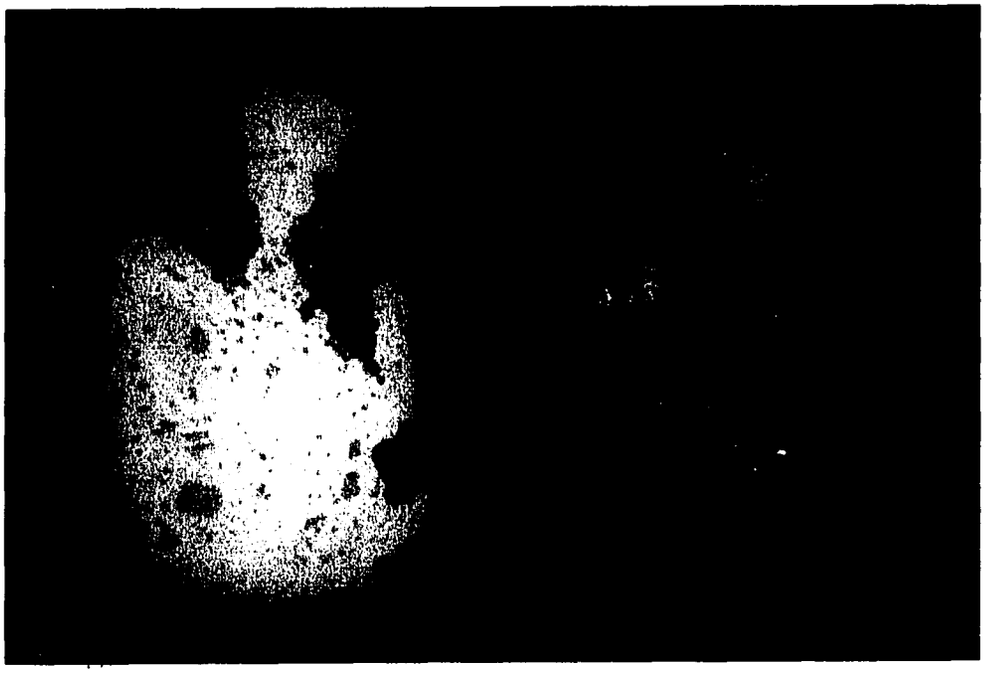


B

Figure 4.10 Original and reduced spotting patterns of *c2-m2* in the presence of *a-m(r)*

A. Original pattern of *c2-m2* is to the right (*c2-m2/c2 wx-844/wx-844 a-m(r)/A*) and suppressed pattern is to the left (*c2-m2/c2 wx-844/wx-844 A/A*).

B. Same as above with wx-mutability shown. Fine wx-mutability at the left indicates the presence of *a-m(r)* and coarse wx-mutability at the right shows the absence of *a-m(r)*. The genotypes are the same as in A



A

B

Figure 4.11 Presence of *a-m(r)* like allele in the *c-m5 5292* fine type of spotting pattern carrying the modifier *En-malt*

A. *c-m/c wx/wx En-malt/- X a/a wx-844/wx.* Presence of colorless and spotted kernels resembling that of *a-m(r)* indicates the presence of an *En* responding allele at the *a* locus

B. *c-m/c wx/wx (No En-malt) X a/a wx-844/wx.* Only colored kernels present indicating the absence of *a-m(r)* allele

C. *c/c En-malt/- wx/wx X a/a wx-844/wx.* Presence of colorless and *a-m(r)* type of spotting pattern present

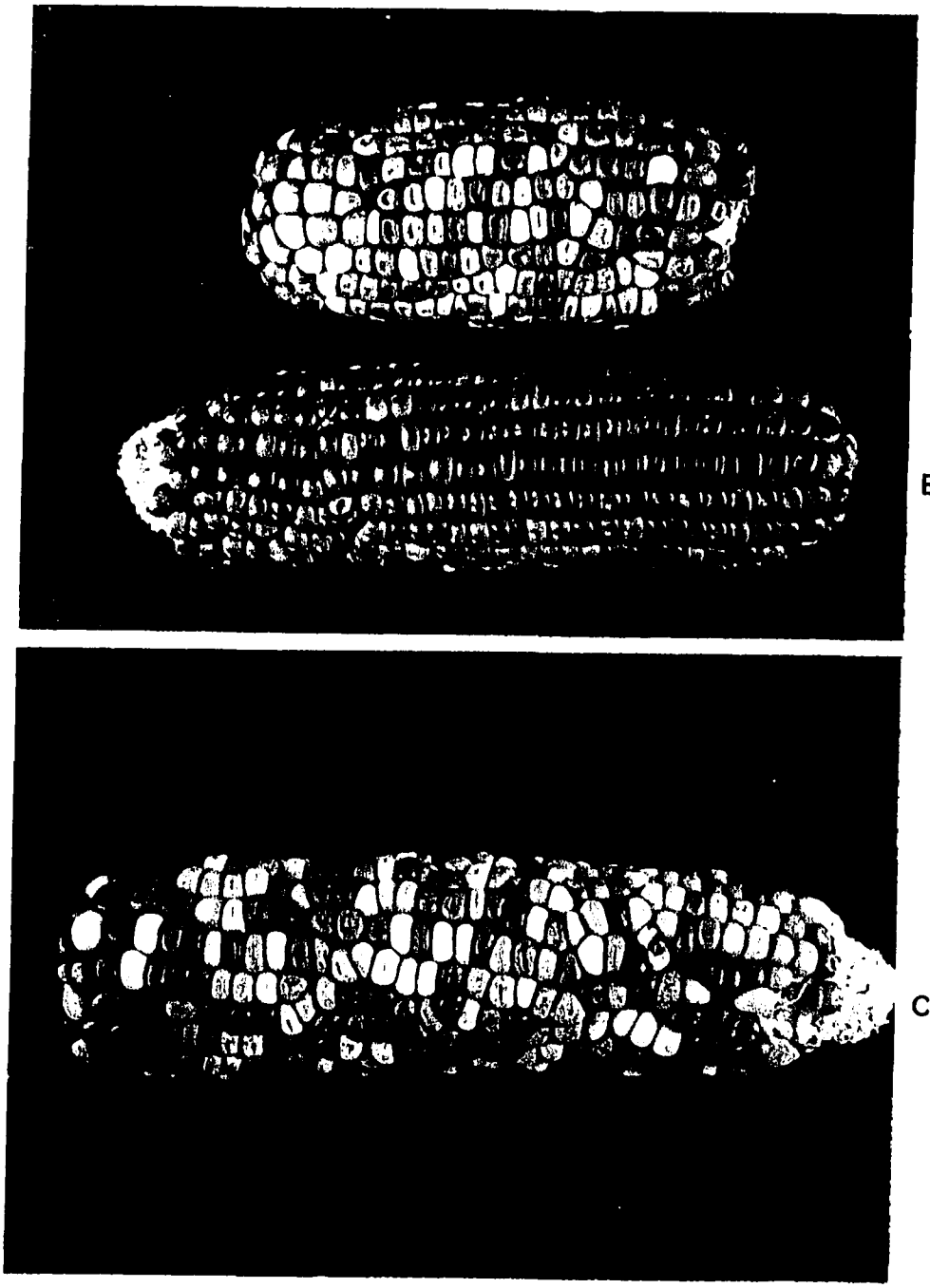
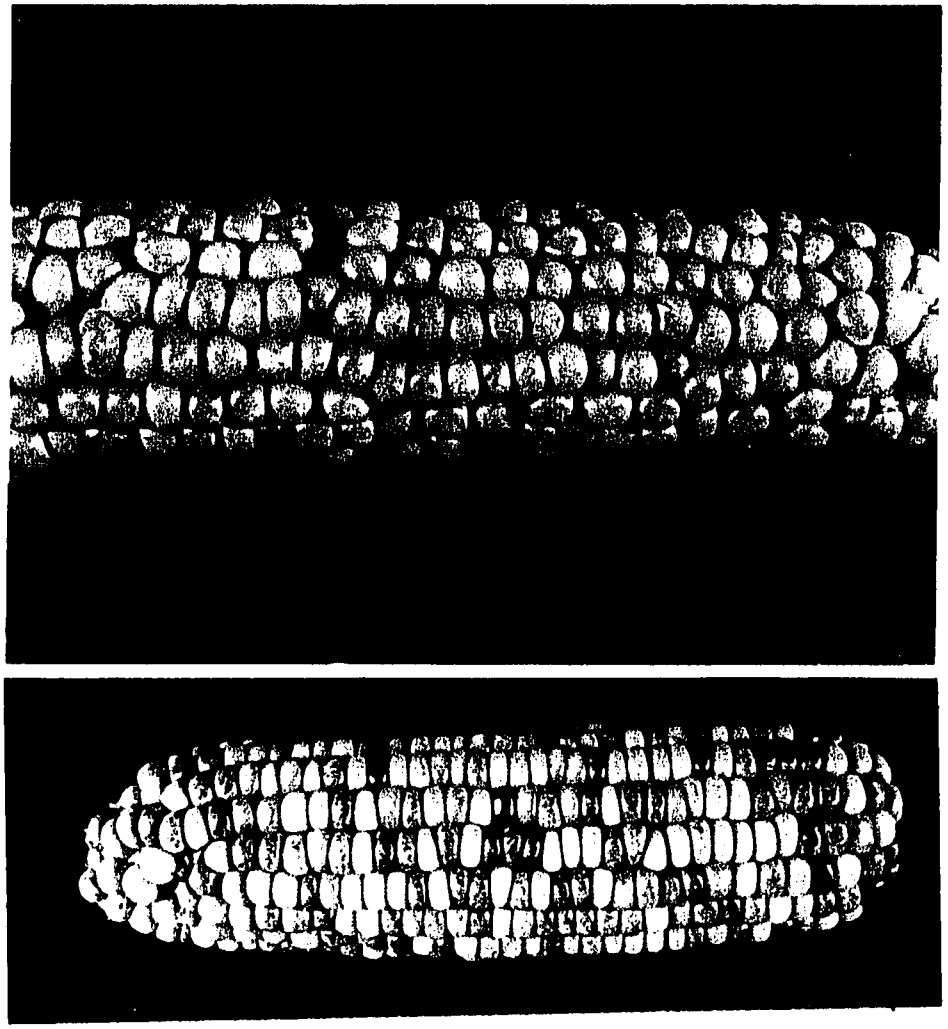

A
B
C

Figure 4.12 Phenotype of a non-suppressing type of *a-m(r)* that was present in a mixed *a-m(r)* tester line

A. Low spotted kernels of non-suppressor type in the presence of *wx-844*. The kernels tested wx-mutable coarse or Wx. Cross: *a-m(r)/a-m(r) wx/wx X a/a wx-844/wx*.

B. The phenotype of *a-m(r) 102* (suppressor type) in the presence of *wx-844*. Note the gigher spotting pattrn. Cross: *a-m(r) 102/a-m(r) 102 wx/wx X a/a wx-844/wx*.



A

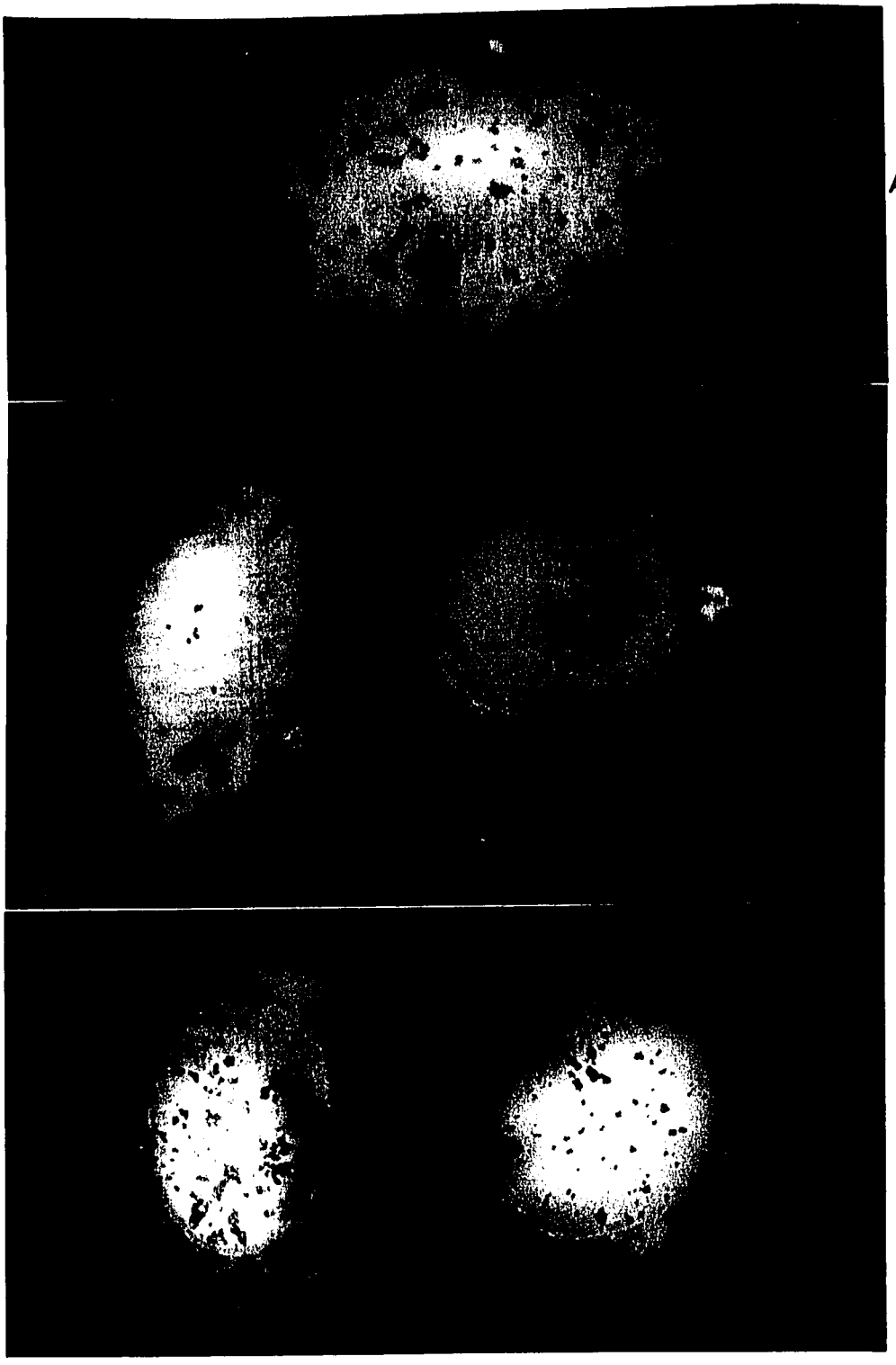
B

Figure 4.13 Dosage effect of *a-m(r)* and *wx-844*

A. 1 *wx-844* 1 *a-m(r)*
B. 1 " 2 "
C. 1 " 3 "
Note the gradual reduction of wx-mutability from A to C.

D. 2 *wx-844* 1 *a-m(r)*
E. 2 " 2 "
F. 2 " 3 "
Note the decrease in wx-mutability from D to F.

G. 3 *wx-844* 1 *a-m(r)*
H. 3 " 2 "
I. 3 " 3 "
Note the reduction pattern of wx-mutability from G to I is not very clear


A
BC
DE


F
G
H I

4.2. Characterization of *wx-m86246X*, a Weak *En* Element

4.2.1. Origin of *wx-m86246X*

This allele was derived from the *wx-844* allele. The *wx-844* allele shows a medium spotting pattern with the *a-m1* reporter allele (Figure 4.15C) and a much higher spotting pattern with the *a-m(r)* allele (Figure 4.1A). In a cross involving the *wx-844* allele and the above reporter alleles, a very low spotted kernel was found as an exception. The expected spotting pattern from the cross:

a-m(r)/a-m(r) wx/wx X *a-m1/a-m1 wx-844/Wx*

should include only high spotted since all the progeny are heterozygous for *a-m(r)* and *a-m1*. The low spotted(1b) exceptional kernel phenotype was heritable when crossed to the *a/a wx/wx* tester (Figure 4.15A). This confirmed that a change has occurred, either in the *En* element present in the line or in one of the reporter alleles involved.

A change in the reporter allele is less likely since, the unchanged *a-m(r)* originated from the parent not containing an *En*. Another possibility is that a very low spotting pattern, atypical of both *a-m(r)* and *a-m1*, could include change in both the alleles of the parental cross to a lower state. The likelihood of this is extremely low.

The second possibility is that the *En* element might have changed to a lower state. This was confirmed by crossing the

exceptional kernel to the *a-m1* tester. The phenotypes that appeared are shown in Table 6. The progeny showed only the low spotted phenotype of the exception even in the presence of unchanged reporter alleles from the tester parent.

The wx-mutability of the kernels from the testcross with *a/a wx/wx* was also very low and agrees with the expectation that *En* has changed to a lower state. The presence of wx-mutability also indicates that the insert is still present at the *wx* locus. The segregation pattern in two similar crosses involving this newly changed allele at the *wx* locus shows that only one *En* is segregating (Table 6. A and B). The weak *En* was also tested for linkage with the *wx* locus in the cross:

a-m(r)/a-m1 wx-m 86246X/Wx X a-m(r)/a wx/wx.

Among the progeny, all the spotted kernels were non-Wx with a very few exceptions. A representative ear is shown in Figure 4.14. The exceptions are likely revertants of the *wx-m 86246X* allele. Eight such ears were tested for tight linkage. These observations collectively, strongly suggest that the new allele at the *wx* locus carries a changed *En* and is weak in its *M* action. This allele is identified as *wx-m86246X*.

Table 6. Segregation pattern of *wx-m 86246X* (Segregation of 1 *En*)

A. Cross: *a-m1 sh/a-m1 sh Wx/Wx* X *a-m(r) Sh/a-m1 sh wx-m 86246X/wx*

Cross	spotted (1-2a-b)			pale/ colored		T	X^2 (1:1: 1:1)
	rd[a]	sh[b]	rd	sh			
86 0542/0246X	59	82		69	57	267	5.88^{ns}

a. some kernels with gown color
b. some kernels are colorless

B. Cross: *a-m(r)/a wx-m 86246X/wx* X *a-m1/a-m1 wx/wx*

Cross	sp	Colored	T	X^2(1:1)
87 5434-3/0904	155	141	296	0.66^{ns}
87 5301/5434-3	204	228	432	1.33^{ns}
87 0906/5434-4	131	108	239	2.21^{ns}

4.2.2. The *S* activity of the weak *En* at *wx-m 86246X*

The original isolate was in combination with two reporter alleles, viz., *a-m(r)* and *a-m1*. The phenotype of this kernel was represented by very few spots on a colorless background (Figure 4.15A). This suggested that this weak *En* has complete *S* activity since the *a-m1* coloration was suppressed. In the testcross ear with *a/a wx/wx*, this observation was also true. However, the test cross ear with the *a-m1* allele showed a large number of kernels with pale coloration in different parts of the kernel, predominately in the gown region (Figure 4:16). This pale coloration is not to be confused with the pale sectors that are seen with a standard *En* and *a-m1* (Figure 4.15C).

Two main types of gown pigmentation were observed with the *a-m1* allele. One had a strong pigmentation on both sides of the embryo (Figure 4.17A) and none on the dorsal side (Figure 4.17B). The other had a strong pigmentation all around the gown region (Figure 4.17D) of the kernel and none at the crown region (Figure 4.17C). Some of these kernels also showed irregularity on the dorsal side of the gown as shown in Figure 4.19A. Kernels were also present with only a small patch of this pigmentation at different parts of the gown. Some of the kernels were completely pigmented except at the very tip of the crown.

Kernels were also present showing colorless areas in the general region of pigmentation and an example of this is shown in Figure 4.18B. The strength of the pigmentation was consistent with the coloration of the *a-m1* allele lacking the *En* and segregating in that ear. For example, the pale coloration in Figure 4.16B and a much stronger pigmentation in Figure 4.17. No kernels were found with pigmentation present at the crown and absent at the gown region.

When the background was pale, rather than strongly colored, some spots were also seen in the pale regions. One example is shown in Figure 4.18B. This feature was not common in all the ears. Some ears had rare kernels with spots in the pale region and other ears had many. These ears also showed relatively higher spotting frequency.

Further, in a segregating ear, not all the spotted kernels showed gown pigmentation. There were always a few which had no gown pigmentation at all.

4.2.3. Dosage of *wx-m 86246X* and its effect on *S* activity

The gown coloration described in the previous section was initially detected in crosses where the *wx-m 86246X* parent served as a male. Very rarely or none were found when the *wx-m 86246X* allele was in two or more doses, i.e., when it originated from the female parent. Kernels obtained from a selfed ear and not showing the gown color were used as both

male and female parents in crosses involving the *a-m1 5719* reporter allele. This allele assays the *S* function of *En*. When used as a female none of the spotted kernels in the progeny showed gown coloration. When used as a male all the progeny ears had kernels showing various degrees of gown coloration. This observation is shown in Table 7A.

In another cross shown in Table 7B the selections were from a cross where the *wx-m 86246X* was used as a male parent. The progeny selected showed gown coloration. But, when they were used as a female parent, none of them showed any gown color as shown in Table 7B.

A further confirmation of this nature was made in combination with the *a2-m1* allele. This allele also assays the *S* function of *En*. Table 8A shows a lack of gown pigmentation when the cross involved the *wx-m 86246X* allele as the female parent (Figure 4.19B). In two cases they were used as male parents and the gown pigmentation appeared among the progeny (Figure 4.19A). Selections from these crosses were made, some having no gown color (Table 8B) and others having gown color (Table 8C). The same trend as mentioned above was also observed. Even though selected parent showed gown color, it disappeared when it was used as a female.

Table 7 Appearance of gown pigmentation with the *a-m1 5719* reporter allele when *wx-m 86246X* is used as male and absence of gown pigmentation when used as female

Cross	male	female
A.		
88 0846Y-1/0632		-
88 0525/0846Y-2	+	
88 0525/0846Y-2	+	
88 0846Y-4/0522		-
88 0632/0846Y-4	+	
88 0525/0846Y-4	+	
88 0524/0846Y-5	+	
88 0524/0846Y-5	+	
88 0846Y-6/0632		-
88 0524/0846Y-6	+	
88 0846Z-1/0524		-
88 0846Z-2/0524		-
B.		
88 0850/0521		-
88 0850/0522(7 ears)		-
88 0850/0632		-
88 0850/0705		-

+: Gown coloration present.
-: Gown coloration absent.
A: Selections were from a selfed ear and were showing no gown coloration.
B: Selections were from an ear having the *wx-m 86246X* from the male parent and were showing gown coloration.

Table 8 Appearance of gown color with the *a2-m1* reporter allele when *wx-m 86246X* is used as a male and absence of gown color when used as female

Cross	male	female
A.		
88 0847-1/3951		-[a]
88 0847-2/3953		-[b]
88 0847-5/0511		-
88 3953/0847-5t	+[c]	
88 0848-2/3953		-
88 0510/0848-2	+	
88 0848-5/3954		-
88 0848-6/3953		-
88 0848-7/3953		-
88 0848-11/0510		-
B.		
89 1013Z/0906		-
89 1013Z/0907		-
89 1013Zt/0906		-
89 1014Y-1/0906		-
89 1014Y-1/0907		-
89 0906/1014Y-2	+	
89 1014Y-3/0906		-
89 1014Y-4/0907		-
C.		
89 1014Z/0907		-
89 0907/1014Z	+	
89 1014Zt/0906		-
89 1014Zt/0907		-

+: Gown coloration present.
-: Gown coloration absent.
A: Selections were of the genotype *A2/a2-m1*. The status of the gown color was not known since, they were all colored
B: Selections were without gown color.
C: Selections were with gown color.
a: Source for the kernels without gown color used in B. as 89 1013Z.
b: Source for the kernels without gown color used in B. as 89 1014Y.
c: Source of the kernels with gown color used in C. as 89 1014Z.

This strong correlation between appearance of gown color and the male parentage of *wx-m 86246X* allele suggests the role of dosage of this weak *En* in its *S* activity. With the maize endosperm being triploid, the male contributes only one dose of the allele and the female contributes two doses. These results suggests that one dose of the weak *En* at the *wx-m 86246X* is not potent enough to suppress the background color of either the *a-m1 5719* or *a2-m1*. At least two doses are needed two express complete and uniform *S* function. This experiment does not exclude the effect of imprinting, as observed with certain *R* alleles (Kermicle, 1978), on the unstable expression of the *wx-m 86246X* allele.

It should be noted that not all the kernels carrying the *wx-m 86246X* allele in one dose show gown color. Some kernels are always present lacking the gown color.

4.2.4. Co-expression of the weak *En* at *wx-m 86246X*

The *wx-844* allele containing a standard *En* shows medium to strong co-expression with the *a-m2 4412* reporter allele (Figure 4.20A, bottom ear). The *wx-m 86246X* in a cross with the *a-m2 4412* allele also shows the same range of co-expression (Figure 4.20A, top ear). Figure 4.20B shows a kernel carrying the *wx-m 86246X* and *a-m2 4412* alleles and reflects a strong pale background. The segregation data in Table 9

Table 9. Segregation pattern of *wx-m 86246X* with the *a-m2 4412* allele (segregation of 1 *En*)

Cross: *a-m2 4412/a Wx/wx* X *a-m(r)/a wx-m 86246X/wx*

Cross	pale w/ and w/o spots	sp w/ cl backgr.	color-less	T	X^2 (2:1:5)
87 6732Y/5434-8t	112	32	301	445	11.92 (5.01)
87 6732Y/5434-10	83	25	182	290	5.01 (.008)
87 6732Y/5434-11	67	25	152	244	1.582

w/ : with
w/o: without

agree with one *En* segregating in the line. This suggests that no other *En* other than the one at *wx-m 86246X* is involved in the strong co-expression shown in these ears recorded.

The range of co-expression in the three ears in Table 9 varied from t1- t4. All three ears were obtained by using the *wx-m 86246X* parent as male. Two of the three plants (5434-10 and 5434 -11) shown in Table 9 were also used as the female parent contributing the *wx-m 86246X* allele. In these two crosses lower co-expression of pale coloration was observed. One ear, shown in Figure 4.21 (bottom) showed t1-t1.5 compared to t1-t4 in the two top ears (Figure 4.21). This is similar to the differences observed in the *S* activity with the *a-m1 5719* and *a2-m1* alleles when *wx-m 86246X* is used as male and female.

4.2.5. The background Wx gene expression of the *wx-m 86246X* allele

The insertion within the *Wx* gene in the *wx-m 86246X* allele does not completely block the expression of the *Wx* gene. This observation was initially made on kernels carrying only one dose of the *wx-m 86246X* allele, i.e., when the *wx-m 86246X* allele originated from the male parent. Faint to strong background Wx staining is prominent among the progeny in crosses when the *wx-m 86246X* allele bearing parent was

used as a male. This background staining is distinct from the wx-->Wx sectors caused by excision of the insert from the *wx-m 86246X* allele. The range of such background Wx expression is shown in Figures 4.22 to 4.24. This Wx staining can not be washed away with hot water washing.

The Wx-like background is not stronger in kernels with the *wx-m 86246X* allele originating from the female parent. This class having more than one dose of *wx-m 86246X* shows a clear to medium strength background. This range is shown in Figure 4.22. Also, most kernels from selfed ears show a very clear background.

Kernels with *wx-m 86246X* coming from the male parent, i.e., 1 dose of the *wx-m 86246X* allele, show a medium to a decidedly very dark Wx background. This range of variability is shown in Figure 4.23 (A-C). Some of them show staining that is indistinguishable from the wild type Wx gene. Nevertheless, the visual phenotype from the unscraped surface of the kernel looks wx instead of the shiny appearance of the Wx phenotype. As mentioned in Section 2.3, there were two types of kernel phenotypes with the *a-m1* reporter allele when the *wx-m 86246X* allele was derived from the male parent. One type showed gown pigmentation and the other lacked it. The kernels with gown pigmentation show darker Wx background (Figure 4.23). When stained in regions showing the background gown color and colorless areas no difference in Wx

background is observed as shown in Figure 4.23C. The kernels having *wx-m 86246X* from the male parent that showed no gown color have weaker background Wx expression. Some of them when stained, showed a very clear background. This is shown in Figure 4.24.

To rule out the possibility that the Wx-like background is not due to some intrinsic property of the genetic stock, a few sib kernels lacking *wx-m 86246X* were always checked. These sib kernels were of the genotype, *a/a wx/wx* and *a-m1/a wx/wx* and were derived from the cross:

a/a wx/wx X *a-m1/a wx-m 86246X/wx*.

None of them showed any background Wx expression. If the background Wx staining were unrelated to the *wx-m 86246X* allele, the sib kernels not carrying the *wx-m 86246X* allele should also have revealed the Wx-like background staining. The above observations suggest that the *wx-m 86246X* allele allows partial expression of the *Wx* gene in spite of the presence of the insert. A likely cause may be read through transcription initiated from the promotor of the *Wx* gene and subsequent splicing of the insert sequence in the matured mRNA.

4.2.6. Presence of twin Wx-wx sectors

Kernels carrying *wx-m 86246X* and showing background Wx also had the wx-->Wx sectors caused by the excision of the insert from the wx locus. They were distinct when the background Wx expression was lighter. Some of these Wx sectors had a very clear non staining wx sector adjacent to them. They give the appearance of twin sectors and are shown in Figure 4.25. The twin sectors are not always of the same size as is expected for all twin sectors. The magnified view of the clear wx areas shows a distinct cellular structure as does the co-twin Wx sectors. This is shown in Figure 4.25 C and D. The observation of cellular structure rules out the possibility that the wx area is due to artifacts caused during scraping of the endosperm. The twin nature of the sectors also strongly suggests their origin being biological rather than artificial. Isolated clear wx areas are also seen among these kernels and are shown in Figure 4.25 C. In some ears the twin sectoring was more frequent than in other ears although the background Wx expression was comparable.

Both the twin sectoring and the wx sectoring indicate that their origin lies in the mechanism of the excision process of the insert.

Figure 4.14 Segregation of the low spotted phenotype of *wx-m 86246X* with the *Wx* locus

In the cross, *wx-m 86246X/Wx a-m(r)/a-m1 X a-m(r)/a wx/wx* most of the spotted kernels have Wx phenotype.

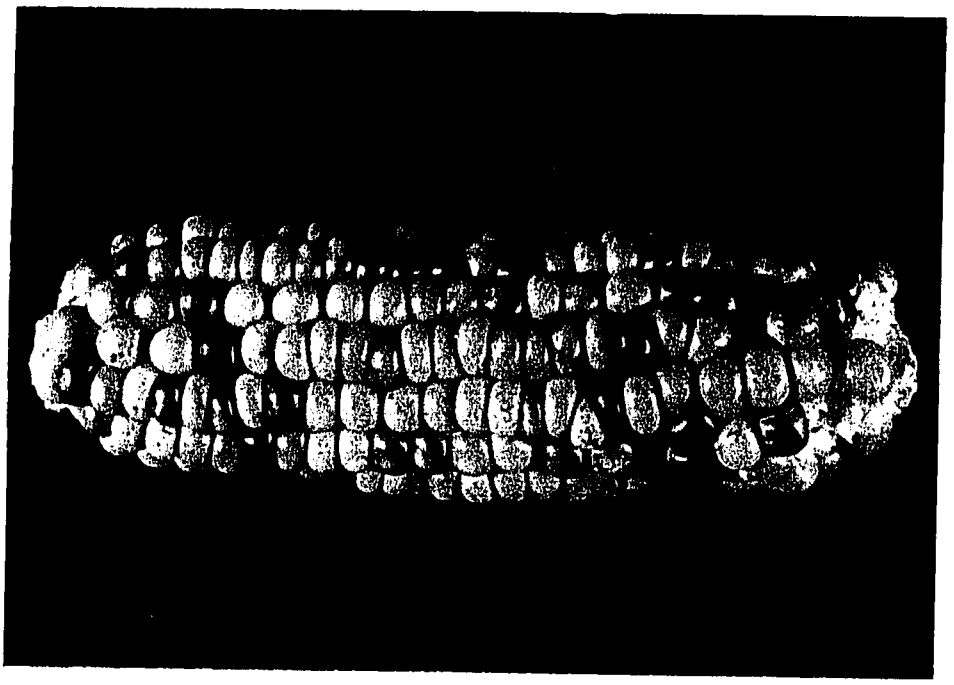


Figure 4.15 Phenotypes of *wx-m 86246X*

A. The low spotting pattern of the *wx-m 86246X* allele with the *a-m1 5719* reporter allele. The low wx-mutable pattern is also shown. The kernel (*wx-m 86246X/wx a-m1/a*) is from the test cross of the original isolate.

B and C. Comparison of the spotting pattern on the *a-m1 5719* reporter allele between *wx-m 86246X* (B: genotype is the same as in A) and *wx-844* (C: genotype, *wx-844/wx a-m1 5719/a*)

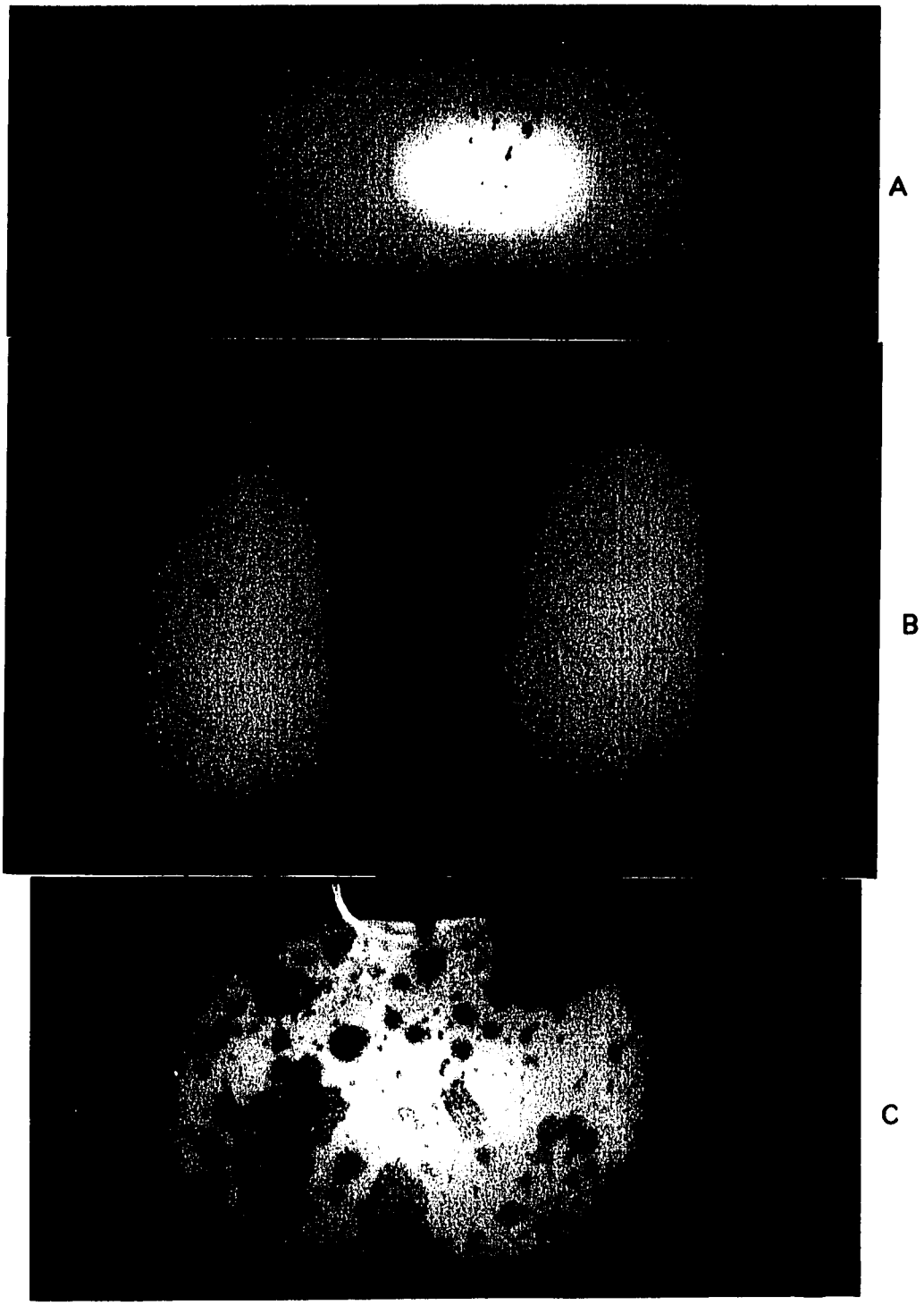
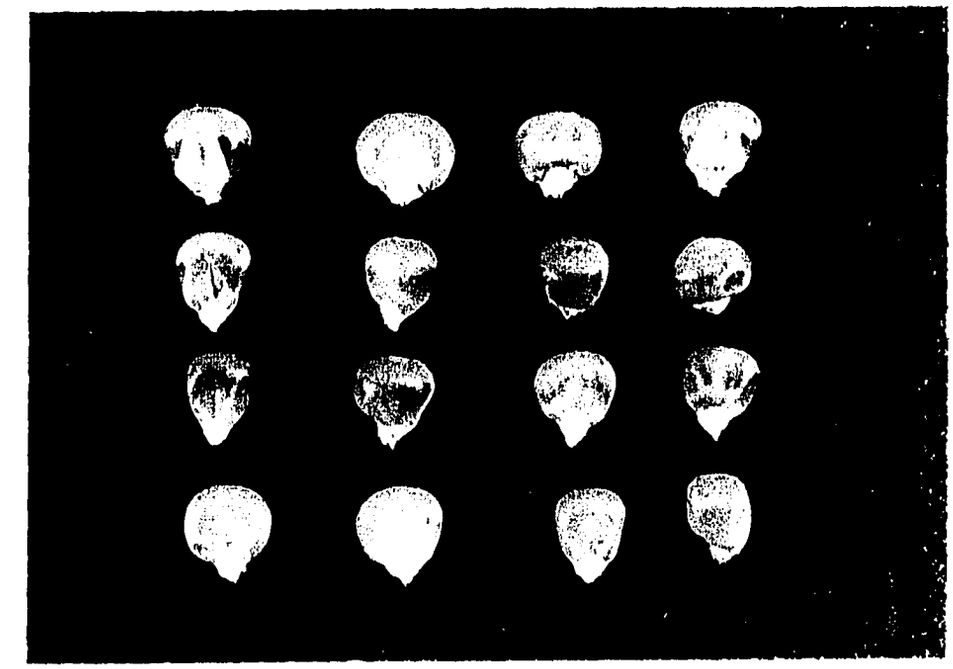

A
B
C

Figure 4.16 Weak *S* activity of *wx-m 86246X*

A. Kernels on the top row show coloraration only around the embryo. The two kernels in the middle show the dorsal side lacking any pigmentation. The second row of kernels have pigmentation in the gown region but none at the crown. The third row of kernels have slightly irregular coloration in the gown region. The bottom row kernels do not have this coloration in any part of the embryo although they are from the same cross (*a/a wx/wx* X *a-m1/a wx-m 86246X/wx*).

B. Two kernels showing pale pigmentation in the gown region. The kernels are of the genotype, *a-m1/a wx-m 86246X/wx*



A

B

Figure 4.17 Lack of *S* activity of *wx-m 86246X* in the gown region

The kernels are of the genotype, *a-m1/a wx-m 86246X/wx* and originated from the cross: *a/a wx/wx* X *a-m1/a wx-m 86246X/wx*.

A. Pigmentation only on both the sides of the embryo.

B. Same kernel as A showing no pigmentation in the back side of the kernel.

C. A kernel showing a lack of pigmentation in the crown region; only spots are present.

D. Same kernel as in C showing the pigmentation is only at the gown region.

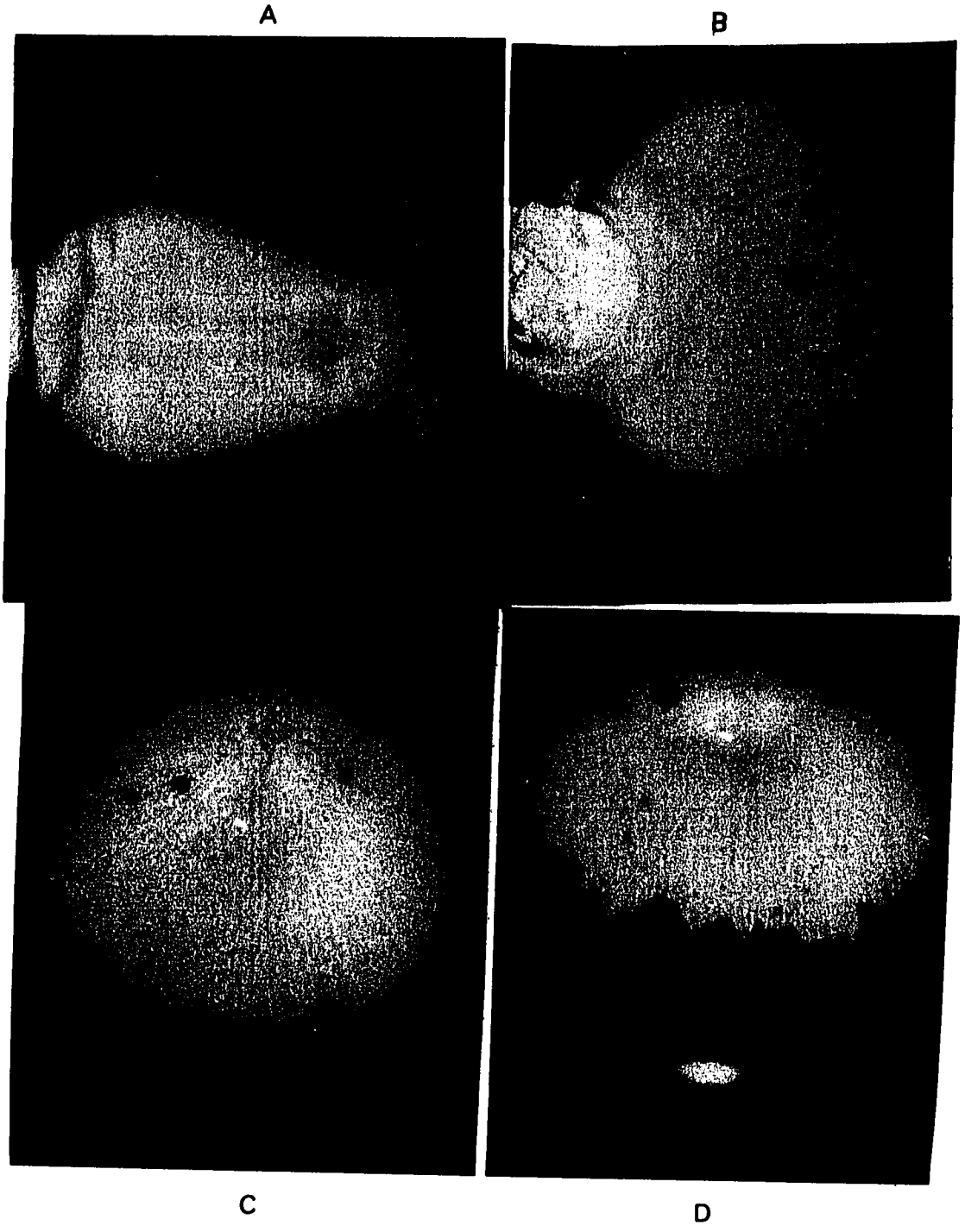
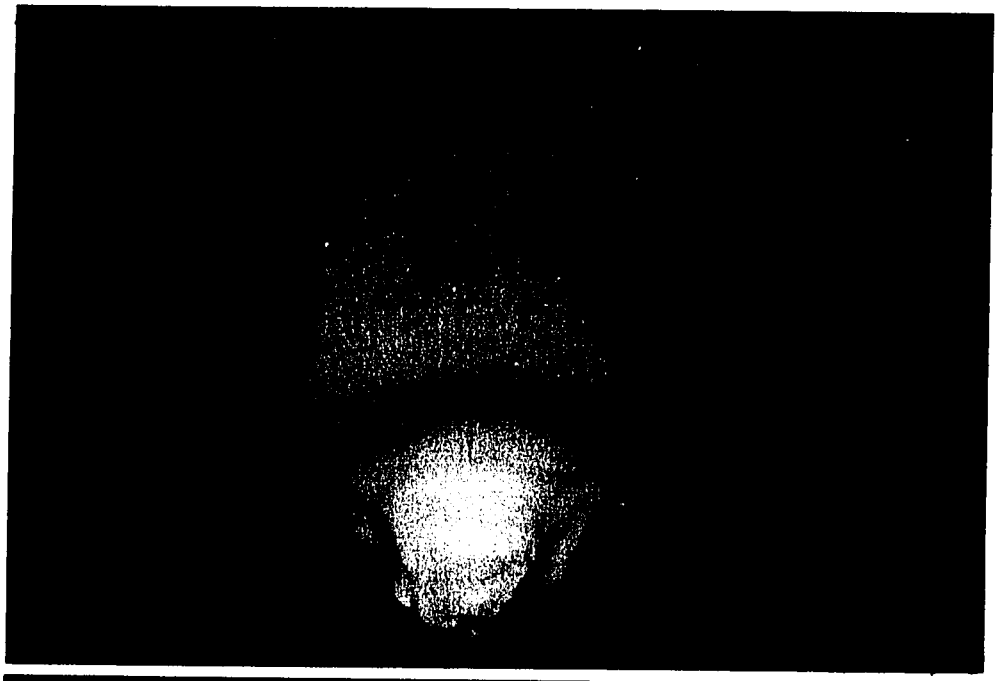

A
B
C
D

Figure 4.18 Weak *S* activity of the *wx-m 86246X* allele

A. A kernel (*a-m1/a wx-m 86246X*) showing irregular coloration in the gown region.

B. One of the occasional kernels (same genotype as in A) that show spots in regions showing the background coloration of *a-m1*.



A

B

Figure 4.19 Expression of the *a2-m1* reporter allele with *wx-m 86246X*

A. *wx-m 86246X* served as the male parent for this ear (Cross: *a2-m1/a2-m1 wx/wx* X *A2/a2-m1 wx-m 86246X/wx*). Note that a number of kernels have no pigmentation at the crown region but have strong pigment expression in the gown region. Some kernels have no pigmentation.

B. For this ear *wx-m 86246X* parent served as the female (Cross: *A2/a2-m1 wx-m 86246X/wx* X *a2-m1/a2-m1 wx/wx*). Note that there is a lack of gown pigmentation in the kernels.

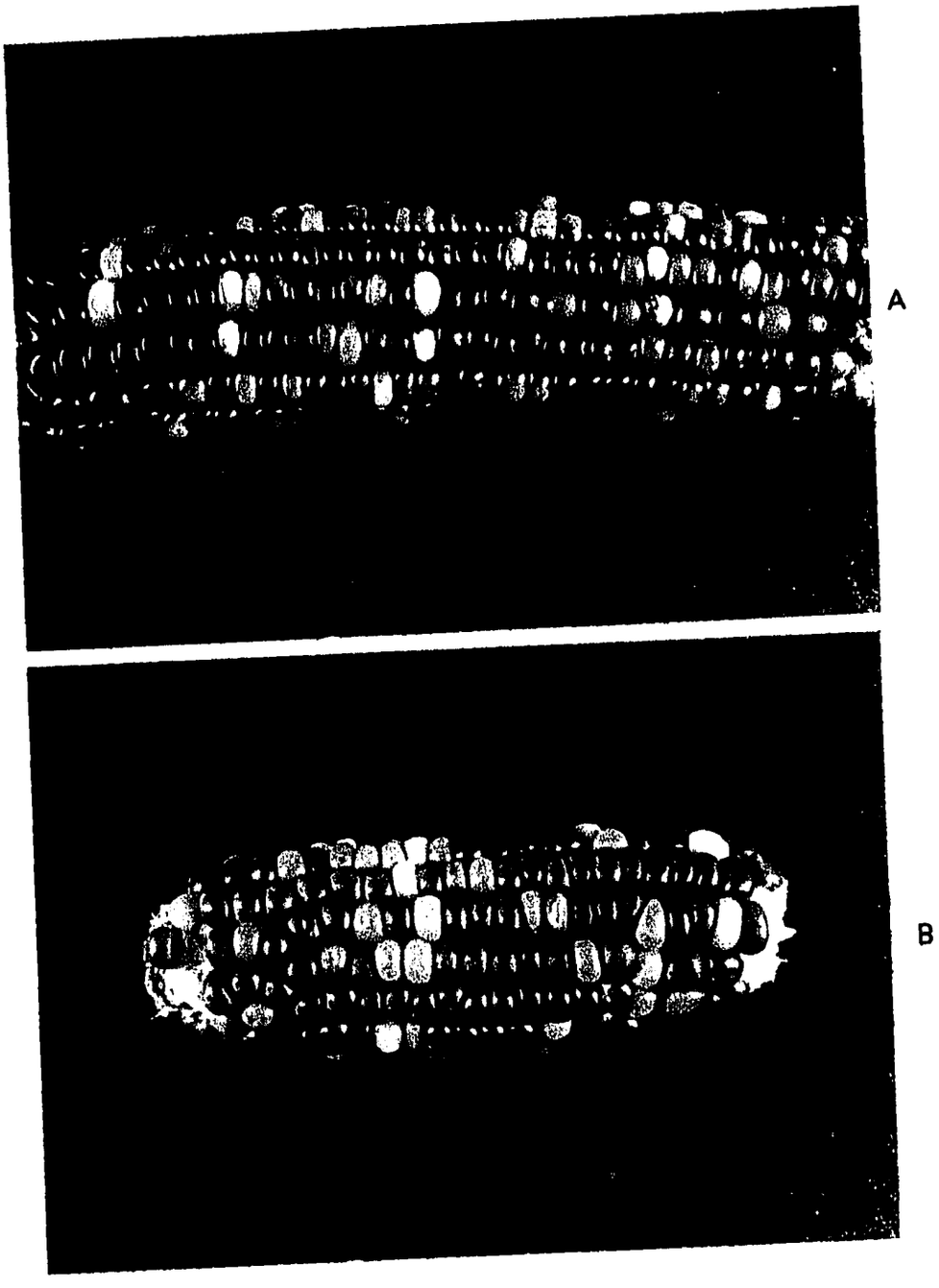
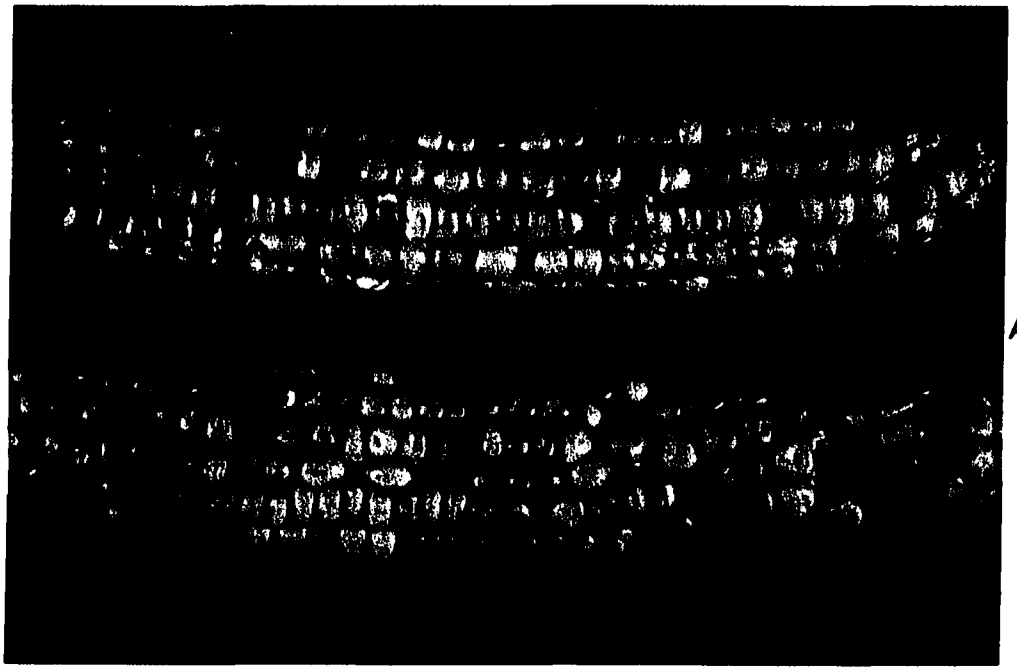

A
B

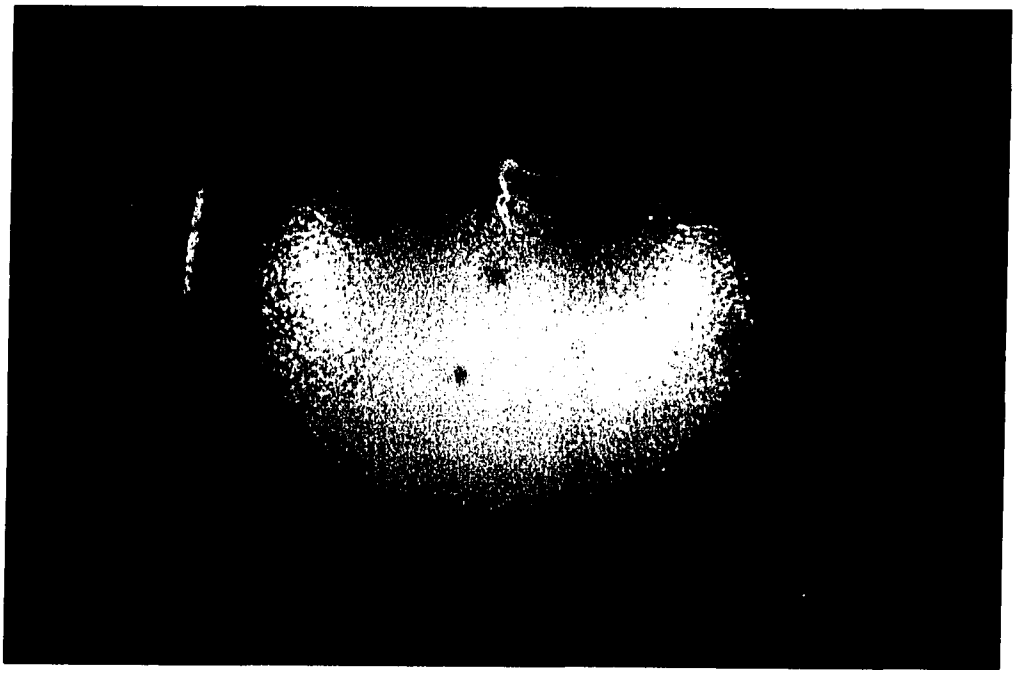
Figure 4.20 Co-expression of the *wx-m 86246X* allele

A. top: This ear originated from the cross, *a-m2 4412/a-m2 4412 or a wx/wx* X *a-m2 4412/a wx-m 86246X/wx*. Note that the kernels are low spotted and also shows the pale co-expression color.
bottom: This ear originated from the cross, *a-m2 4412/a-m2 4412 or a Wx/wx* X *a/a wx-844/wx*. Note that the kernels have a higher spotting pattern compared to those in the top ear. This ear also shows that the range of co-expression of the *wx-844* allele is comparable to that of the *wx-m86246X* allele in the top ear.

B. A kernel (*a-m2 4412/a-m2 4412 or a wx-m 86246X/Wx*) showing lower spotting pattern and strong co-expression. The strong pale background color indicates that the co-expression capacity of *En* is retained in the *wx-m 86246X* allele



A



B

Figure 4.21 Co-expression of *wx-m 86246X* allele when used as male and female

Top and middle: Thes two ears (cross: *a-m2 4412/a-m2 4412 or a wx/wx* X *a-m2 4412/a wx-m 86246X/wx*) show stronger co-expression than the bottom ear (cross: *a-m2 4412/a wx-m 86246X/wx* X *a-m2 4412/a-m2 4412 or a wx/wx*)

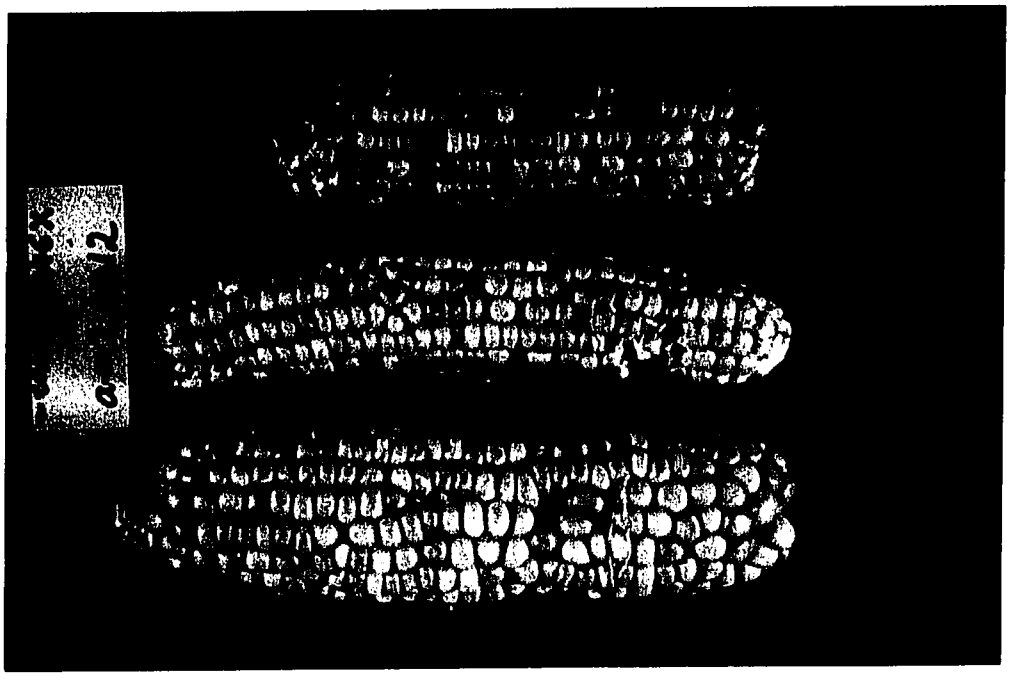
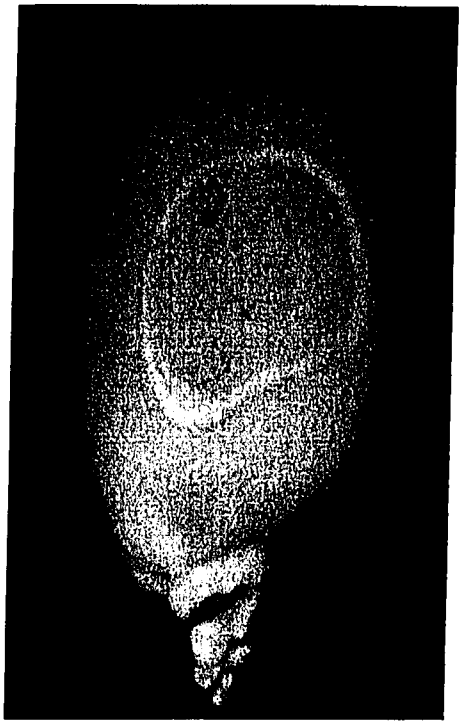
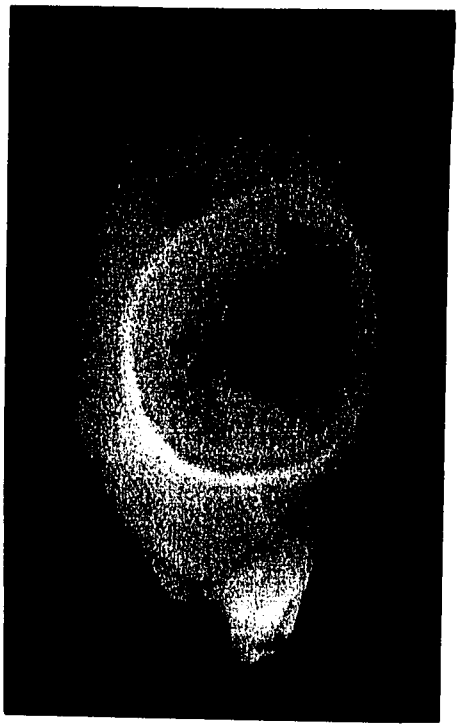


Figure 4.22 **Background expression of the *Wx* gene in *wx-m 86246X***

Thes kernels are obtained from a cross where *wx-m 86246X* originated from the female parent. The background expression of the *Wx* gene varies from none to some expression. The kernels are of the genotype, *a-m1/a wx-m 86246X/wx*





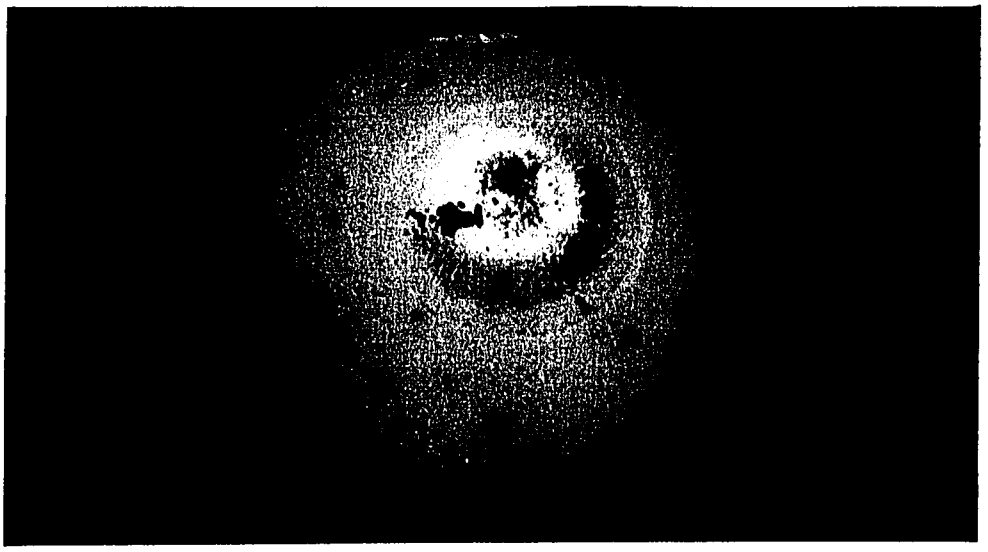


Figure 4.23 Background expression of the *Wx* gene in *wx-m 86246X*

The kernels are of the genotype, *a-m1/a wx-m 86246X/wx*.

A and B. These kernels originated from the cross where *wx-m 86246X* carrying parent served as a male. Note the stronger background expression of the *Wx* gene than that shown in figure 4.22.

C. A kernel showing that there is no difference in the *Wx* expression between the pigmented region and the nonpigmented region. Note that there is background pigmentation in the gown region in all the kernels.

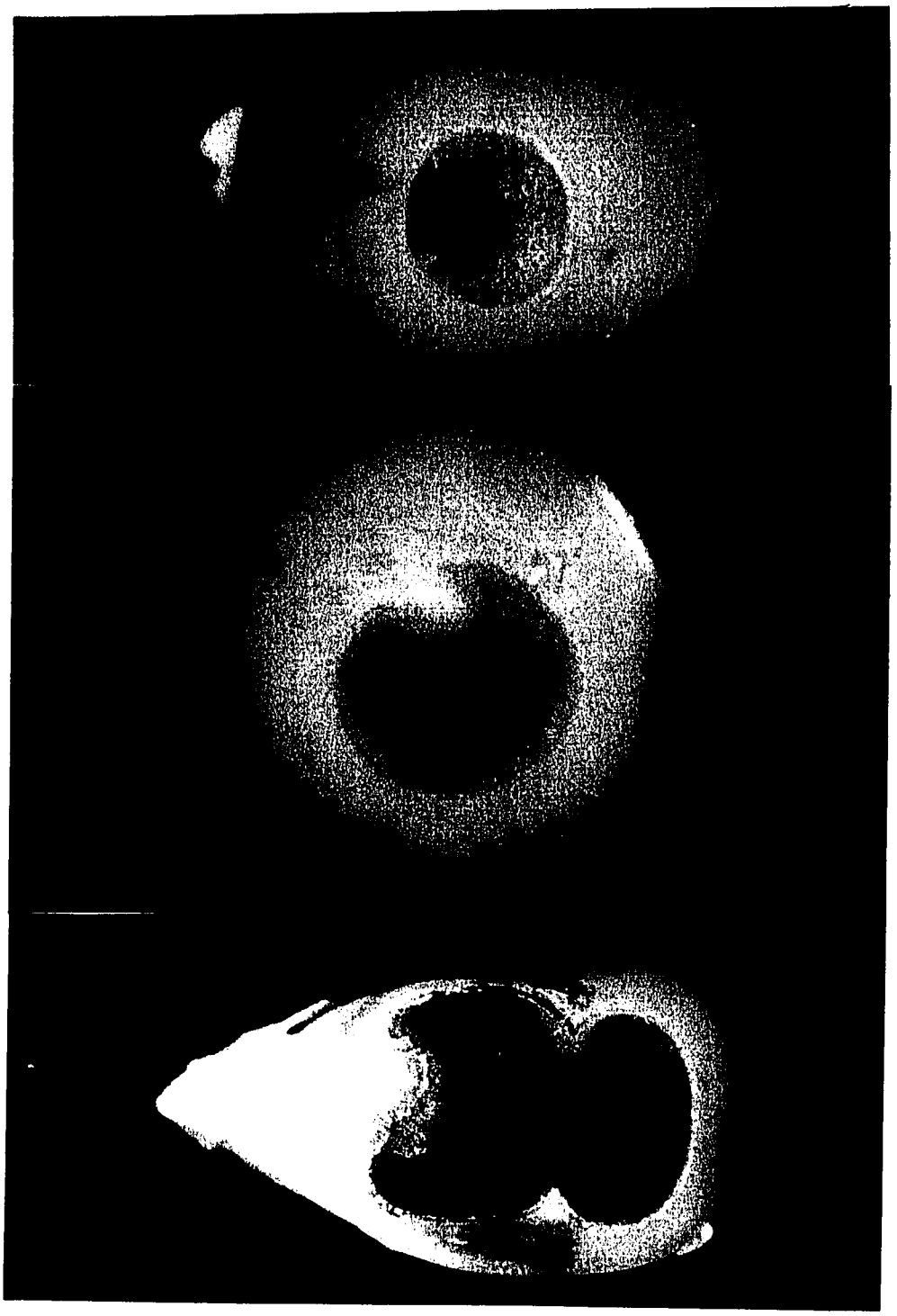

A
B
C

Figure 4.24 Background *Wx* expression when *wx-m 86246X* originated from the female parent

The kernels are of the genotype, *a-m1/a wx-m 86246X/wx*.

Note the lack of *Wx* expression in A and some background in B. These kernels do not show the background pigmentation in the gown region.

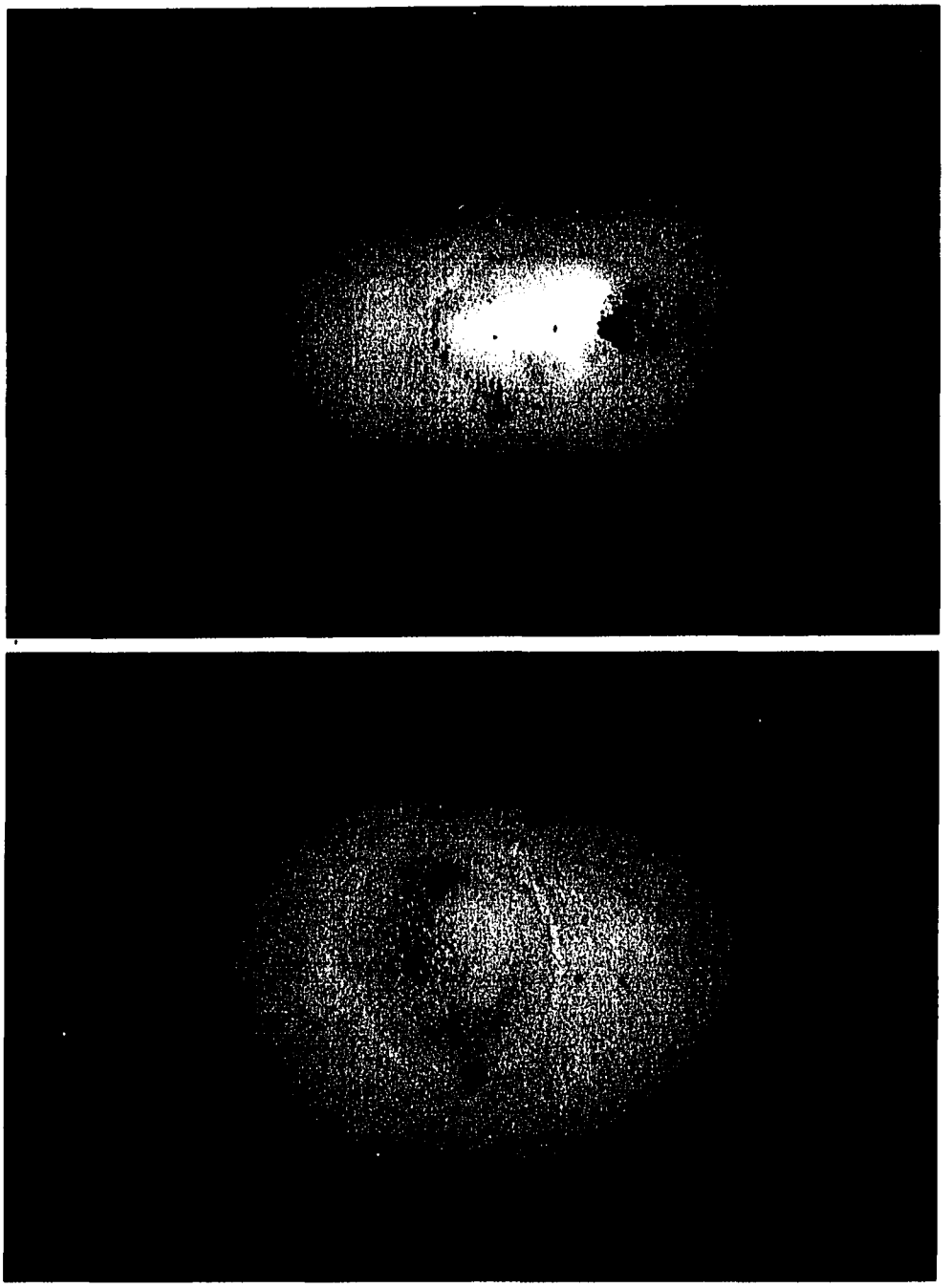
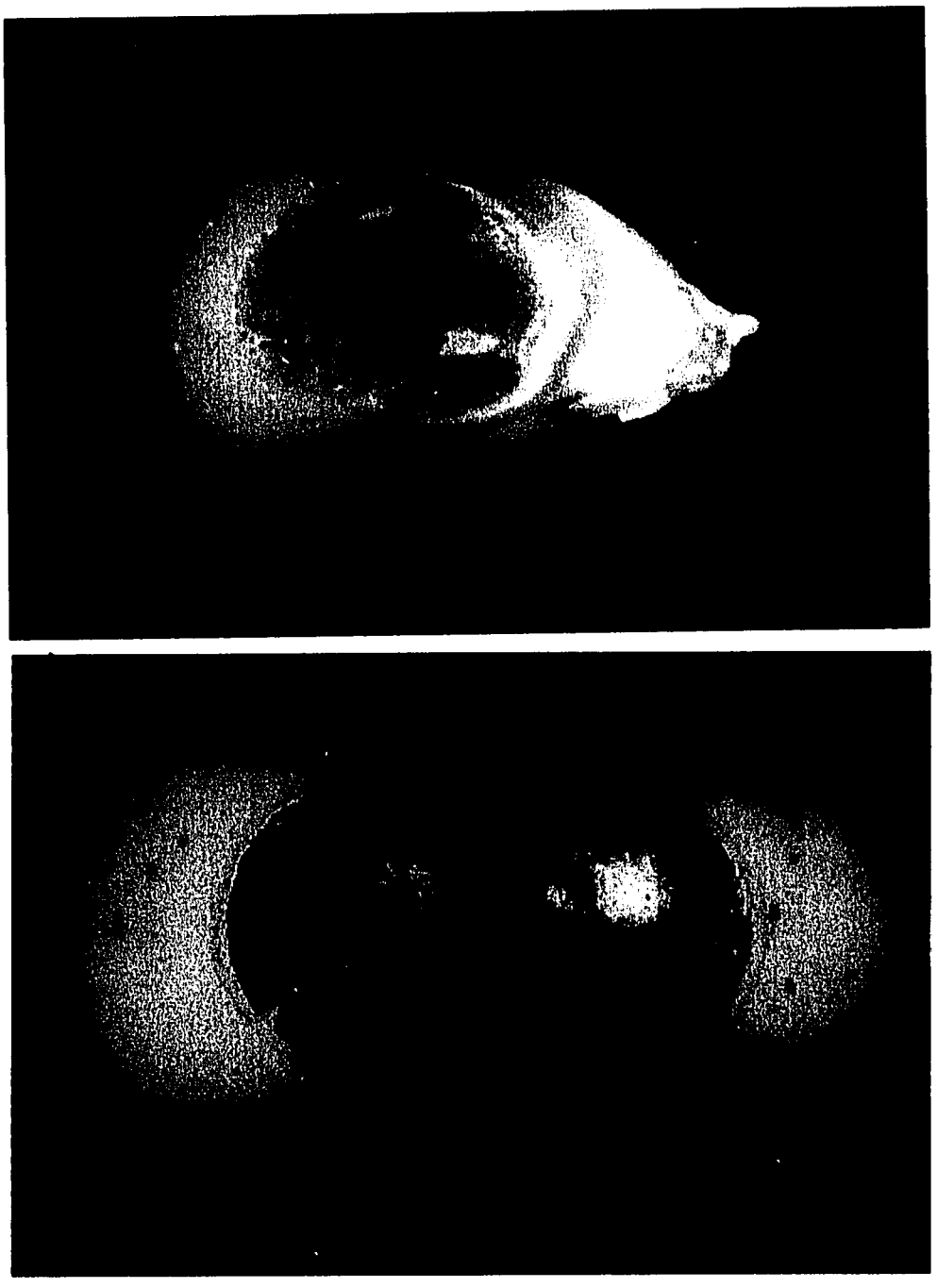

A
B

Figure 4.25 Twin sectoring seen with the *wx-m 86246X* allele

Wx-wx twin sectors are observed in a light to dark Wx background when the wx-m 86246X parent is used as a male. Note the size of the *Wx* and *wx* co-twin sectors are not be equal. The magnified view in C and D shows the cellular structure of the twin sectors. The kernels are of the genotype, *a-m1/a wx-m 86246X/wx*



A

B

C

D

4.3. Somatic Events Observed with the *wx-844* Allele

Excision and transposition of *En* can be observed both somatically and germinally, provided, there is a suitable reporter allele. Germinal events can be recovered if the event takes place in a tissue that terminates in the production of either the female or in the male gametes. These germinal events are seen as exceptional kernel phenotypes when compared to the expected phenotype of the rest of the progeny and further, they have the advantage of being available for further genetic analysis.

Somatic events, on the other hand are easily visible in a background of the expected phenotype. There is a disadvantage in that they can be interpreted but not confirmed unless a similar event is isolated germinally and is available for testing. This section deals with observations made on somatic events.

Because the maize endosperm is triploid, the number of copies of *En* in the endosperm can be adjusted. This can be augmented by providing the *En* from the male or female parent since the male parent contributes one dose and the female parent contributes two doses of the corresponding allele. To properly study the excision process, the ideal condition is to have the *En* in only one copy, i.e., to use the *En* source as the male parent. This facilitates the detection of any change that may occur during the excision process of the

single *En* in the test. Presence of extra *En*(s) will obscure changes identified with a single *En*. This argument has been used in the subsequent sections and has also been used in constructing genotypes for studying the excision process.

4.3.1. Somatic events observed with the *wx-844* and the *a-m(r)* allele

The *a-m(r)* allele shows a high spotting pattern in the presence of *En* as shown in Figure 4.1. The spots are generally small (late events) and uniformly distributed on the aleurone. Variation in the frequency of spotting is also observed, and may be due to different doses of either *a-m(r)* or *wx-844* or both or due to the difference in the genetic background. Occasionally, larger colored sectors are also observed due to early excision of the *I* element of the *a-m(r)* allele. The above features describe the phenotype observed with the *a-m(r)* allele when *En* is present in more than one dose.

The following observations were made from kernels obtained from one of the crosses listed below.

a/a wx/wx X *a-m(r)/a-m(r) wx-844/wx-844*; and

a/a wx/wx X *a-m(r)/a wx-844/wx*.

In both crosses the *wx-844* and the *a-m(r)* alleles originated from the male parent. Thus all the kernels among the progeny maintained only one dose of *En* (*wx-844*) and one dose of the *a-m(r)* reporter allele. In addition to the fea-

tures described in the beginning of this section, distinct low spotted and colorless sectors are also observed in a background of high spotting (Figure 4.26 A and B). The various identities of the wx-mutability patterns underneath these sectors are summarized in Table 10 and are shown in Figures 4.27 and 4.28. The aleurone pigmentation of the sectors and the wx-mutability in the underlying endosperm provides clues to the origin of such sectors. The plausible events that may cause the different types of sectors are also summarized in Table 10.

Changes at two levels can affect the genetic composition of the sector. A change in the *a-m(r)* allele will affect the spotting pattern on the aleurone and may also affect the expression of the coarse or fine pattern of wx-mutability in the endosperm. Similarly a change in the *wx-844* allele will affect the wx-mutability pattern in the endosperm and may also affect the spotting pattern on the aleurone that is dependent on the fidelity of the *a-m(r)* allele.

Individual sectors that have retained the *wx-844* allele in that sector are described here. All these sectors (Table 10, #s 4, 7, and 10) are wx-mutable coarse in the endosperm and therefore indicates that an intact *wx-844* allele is present. The spotted low, wx-m coarse (Table 10, #4) sector indicates that the *a-m(r)* allele has changed to a lower state and has also lost the ability to repress excision. The colored wx-m coarse sector (Table 10, #7) originated as a

result of excision of the *I* element from the *a-m(r)* allele. Lack of suppression of the coarse wx-mutability in this sector suggests that the *I* element is lost after excision. The colorless sector that shows a wx-m coarse phenotype in the endosperm (Table 10, #10) has its origin also in the excision of the *I* element from the *a-m(r)* allele. In this case the excision of the *I* element resulted in a non-responsive *a-nr* allele and is therefore, colorless in the aleurone. It is concluded here that low spotted, colored, and colorless aleurone can be ascribed to a change in the *a-m(r)* allele.

Aleurone sectors that are spotted low, colored, and colorless and are wx-m fine in the endosperms have also been observed (Table 10, #s 3, 6, and 9, respectively). The colored and colorless sectors in this class can arise due to excision of the I element and subsequent reinsertion, thus retaining the suppressing ability on the wx-mutability pattern. The spotted low phenotype (#3) could arise either due to a change in the *a-m(r)* allele or a change in the *En* at the *wx-844* allele. A change in the *a-m(r)* allele to a lower state yet retaining the suppressing ability can lead to the above phenotype. A change in the *En* to a lower state would also result in a low spotted and finer or lower mutability pattern. These observations also suggest that the altered aleurone spotting patterns can arise due to a change at the *I* element as in the previous group (Table 10, #4, 7, and 10).

The Wx sectors are of particular importance from the point of view of determining the destiny of the excised *En* from the *wx-844* allele. Of the eleven somatic events observed and listed in Table 10, only three events are phenotypically Wx and therefore, are products of excision. These are numbers 2, 5, and 11 listed in Table 10. The aleurone patterns of these Wx sectors are spotted high (#2), spotted low (#5), and colorless (#11) (Figures 4.28A, 4.28B, and 4.27A respectively). Only rarely, is there the concomitant excision of both *En* and *I* (#8) resulting in a colored Wx sector. The spotted high Wx sectors (Table 10, #2; Figure 4.28A) are caused by the transposition of *En* from the *wx-844* allele. The excision of *En* from *wx-844* causes Wx, yet the presence of spotting suggests the presence of *En* in that sector, i.e., that *En* is excised from the *wx-844* allele (the Wx phenotype) and is reinserted elsewhere. The spotted low Wx sector (Table 10, #5; Figure 4.28B) is also an outcome of the excision event at *wx-844*. The low spotting may either be due to a change of state of *En* following transposition or a concomitant change in the *a-m(r)* allele. The low spotting can not specifically be ascribed to alterations in the *En* element because it has earlier been noticed that the *a-m(r)* allele can change in somatic sectors.

The third type of sector caused by an excision of *En* from the *wx-844* allele is colorless (Table 10, #11; Figure 4.27A) in the aleurone and suggests a lack of *En* in that

sector since the *a-m(r)* allele shows a colorless aleurone in the absence of *En*. The loss of *En* following excision can occur if the excised *En* is not reinserted or if reinsertion occurs following chromosome replication such that *En* is reinserted in the sister chromatid. This will cause one sister chromatid to lack the *En*. The segregation of this chromatid lacking the *En* into a cell will cause a colorless aleurone.

Other events can also cause a colorless aleurone sector and Wx endosperm. *En* may change to a very low state following transposition from the *wx-844* allele. Such as the low state (rare spots on the aleurone) of the *wx-m 86246X* allele described in Section 4.2. Thus in a sector, the presence of such an *En* causing rare spots may give the appearance of a colorless sector. Another explanation is that a change of *En* to *S+M-* state will also cause a colorless aleurone. The *a-m(r)* reporter allele is not capable of revealing the *S* function of *En*. Therefore, it is not possible to distinguish an altered *En* that has a changed *M* and an intact *S* function from the loss *En*. Both, loss of *En* and S+M- state *En* will make the aleurone colorless in the presence of the *a-m(r)* reporter allele.

In summary, three outcomes can be recognized after *En* has excised from the *wx-844* allele: 1. reinsertion with no change of *En*, 2. reinsertion with a changed phenotype that may be attributed to either a change in the transposed *En* or

a change in the *a-m(r)* reporter, and 3. an apparent lack of the M function of *En* after excision.

From the events analysed in this section, it is concluded that the *a-m(r)* reporter allele is not very suitable to study the destiny of *En* in somatic tissue. This is recognized from two behaviors of this reporter: 1. the *a-m(r)* allele shows frequent changes in the somatic tissue in the presence of *En* and therefore, the cause of the observed sectors cannot unambiguously be attributed to events originating at the *En* element, and 2. the *a-m(r)* allele detects only the M action of *En* and therefore is not helpful in distinguishing loss of *En* from *Ens* with altered M activity.

The following section makes use of a reporter allele with which, the S function of *En* can also be monitored along with the M function in somatic sectors.

4.3.2. Somatic events observed with the *wx-844* and the *a-m1 5719* allele

The *a-m1* allele shows a strong pale to colored aleurone phenotype in the absence of *En*. In the presence of *En*, spots of a medium frequency appear and in addition, various sized pale sectors are also seen (Figure 4.29A). The colored spots and pale sectors are distinguishable. The spots caused by excision of the *I-5719* from the *a-m1* allele are darker than the pale sectors. The pale sectors however can appear very pale to dark depending on the coloration of the *a-m1* allele

segregating in that ear. The nature of this variation in the pale coloration is not known. When the *wx-844* allele is present in three doses, i.e., homozygous, the pale sectors are rarely seen. When it is present in two doses, these pale sectors are reduced in size and are also less in number. In contrast, with one dose of *wx-844* a greater number and larger sized pale sectors appear. The *a-m1* reporter allele is pale in the absence of *En* due to a lack of the *S* function of *En*. Therefore, the pale sectors reveal regions lacking the *S* function of *En*. Distinguishably darker spots are caused due to the *M* function of *En*. Types of sectors seen with one dose of *wx-844* are listed in Table 11.

When large sized Wx sectors are considered, with this genotype two types of spotting patterns are observed in the aleurone overlying them. One type of Wx sector (Table 11, #2) is spotted with pale sectors in the aleurone. These spots are caused by the excision of *En* from *wx-844* and reinsertion elsewhere, i.e., a somatic transposition event. The second type is a pale coloration of the *a-m1* allele overlying the Wx stained endosperm sector (Figure 4.29B). The most likely event is the loss of *En* after excision from the *wx-844* allele. When pale sectors are examined, they always show Wx endosperm underneath. Some are part of a large Wx staining sector while others match margin to margin with the Wx sector. No exception to this observation has been observed. Therefore, all the pale sectors are caused by an excision

event of *En* from the *wx-844* allele, since they are always associated with Wx staining endosperm. The pale coloration of the sectors is indicative that there is no activity of the *S* function of *En*. This issue was unresolved in the previous section where the *a-m(r)* reporter allele was used. The absence of spots in these sectors also indicates the absence of the activity of the *M* function of *En*. The absence of both functions in the same sector is the same as the absence of *En*. The phenotype of *a-m1 5719* with one dose of *En* shows a substantial number of pale sectors and indicates that the loss of *En* occurs quite frequently, at least somatically.

A significant feature of these large pale sectors is the appearance of smaller colorless regions within the pale sectors (Figure 4.29A). A colorless aleurone with the *a-m1* allele implies the presence of *En*. It is difficult to comprehend the appearance of *En* inside a sector in which the *En* is assumed to be lost, i.e. not reinserted after excision. A likely reason is the incorporation of adjacent colorless tissue during development of the endosperm within the developing colored tissue.

It has already been noted earlier in this section that the number of pale sectors are fewer and smaller in size when the dosage of *En* is increased from one to two. Also three doses of *En* severely restricts the appearance of pale sectors. This dosage effect on the number and size of pale sectors agrees with a model that supports the loss of *En*.

The alternative possibility that En may be inactivated in the sectors showing the pale color is considered in the discussion section.

4.3.3. Somatic events observed with the *a-m2* allele in the presence of one dose of *wx-844*

The *a-m2 8004* and the *a-m2 4412* alleles show a colorless aleurone in the absence of *En*. In the presence of *En* the *a-m2 8004* allele shows spots due to excision of the *I* element and in addition ringed areas with strong pigmentation in the border region (Figure 4.30A). The *a-m2 4412* allele shows varied degrees of pale pigmentation and occasionally, colorless sectors in the presence of *En*. The relationship between the colorless and the ringed areas and the excision of *En* at the *wx-844* allele is revealed from the nature of the Wx staining in the endosperm underlying these sectors.

In the case of the *a-m2 8004* allele, the ringed areas (Figure 4.30A) are always found to be Wx stained underneath (Figure 4.30B). When the surrounding area is wx-mutability the ringed area completely matches the Wx sector in the endosperm, border to border (Figure 4.30B). Some of the ringed areas are also part of a larger Wx sector. Similarly, the colorless areas are also always Wx in nature. Some colorless areas are part of a bigger Wx sector. The Wx nature of these sectors suggests that their origin is always associated with an excision event at the *wx-844* allele. The colorless

nature of the inside of the ringed areas suggests that the *En* is no longer active in that sector.

There are no spots that can be ascribed to excision that are found inside the ringed areas. Occasionally, within a large ring a smaller ring is also found. They are more often found near the border and some even appear as an invagination of the border of the ring into the internal part of the ring. These observations suggest that smaller rings inside larger ones are inclusions of a group of cells that are originally not part of the ring. This event might have occurred during rapid cell growth of the endosperm tissue. Very small rings sometimes appear as spots, although they could be rings with their rims very close such that they appear as a single spot. The origin of the rings are due to the loss of *En* following excision and this explains the observations with the *a-m2* alleles.

4.3.4. Somatic loss of *En* observed with the *a2-m1* allele

The *a2-m1* allele shows a fully colored phenotype in the absence of *En* and a sectored phenotype in the presence of *En*. the number of *En* present in the genome also determines the nature of spotting observed with this allele. A single *En* in the genome causes a coarse pattern of sectoring (Figure 4.31). The coarse pattern consists of colored sectors of various sizes that are frequent in number. With two doses of *En*, a fine type of sectoring pattern appears. This pattern shows smaller spots and also a reduced number of spots in the

aleurone. Increased doses of *En* causes the pattern to become more fine and appears to be colorless.

Kernels carrying only one dose of *En* (*a2-m1/a2-m1 wx-844/wx*) were examined to determine the nature of the colored sectors. A colored sector whenever examined, was always Wx staining in the endosperm. No colored sectors were found with wx endosperm below them. In some cases the colored sectors were part of larger Wx sectors and in other cases the Wx sectors and the colored sectors were matching margin to margin. Since the *a2-m1* allele shows a colored phenotype in the absence of *En*, the colored sectors obviously lacked *En* activity. The Wx nature of the endosperm suggests that the events are associated with excision of the *En* from the *wx-844* allele. The most likely event is the loss of *En* in that sector after excision from the *wx-844* allele.

The appearance of colorless regions within pale sectors observed with the *a-m1* allele has been described in the previous section. Similar instances of colorless regions are also observed within the colored sectors. Incorporation of adjacent colorless tissue into the colored sector is the most likely cause of this pattern.

In general the *wx-844* allele shows a substantially high degree of loss of *En* after excision. The types of sectors observed provide clues to the cause of this phenomenon and is described in the next section.

4.3.5. Association of *En* loss event with replication (Analysis of twin sectors)

The *a2-m1* reporter allele was found suitable for this purpose because it can distinguish different doses of *En*. Secondly, it does not respond to the *M* function of *En*. In the absence of a response to the *M* function, this reporter allele is a fixed entity and is not subject to any change caused by the transactive functions of *En* (McClintock, 1958; Menssen et al., 1990). Therefore, alterations observed in the spotting pattern can not be due to a change in the reporter allele. This was not possible with the two reporter alleles, the *a-m(r)* and the *a-m1* alleles, used previously. This advantage is unique to the *a2-m1* reporter allele and simplifies the interpretations of observations to events caused by the *En* element at the *En* containing *wx-844* allele.

All the observations were made on kernels carrying only one dose of *En* (*a2-m1/a2-m1 wx-844/wx*), i.e., kernels showing a coarse spotting pattern, and they were also obtained from ears segregating for only one *En*. They were derived from the cross: *a2-m1/a2-m1 wx/wx* X *a2-m1/a2-m1 wx-844/wx*. The only *En* in question here is the one carried at the *wx-844* allele that originated from the male parent.

Coarse spotted wx-mutable kernels were observed for different types of sectors in the aleurone and in the endosperm. The types of sectors observed are summarized in Table 12 and Figure 4.35 represents a diagrammatic view of the

sectors observed. These observations are on individual sectors. The most obvious sectors are the colored (Table 12, #5), and fine (Table 12, #3, #4) sectors in the aleurone. They are easily visible in a background of coarse spotting. When the kernels were scraped for endosperm staining, the colored sectors were always Wx (Table 12, #5; Figure 4.35, #5) and the fine sectors were either wx-mutable (Table 12, #3; Figure 4.35, #2) or Wx (Table 12, #4; Figure 4.35, #4). The coarse spotted regions were either wx-mutable (Table 12, #1; Figure 4.35, #1) or Wx (Table 12, #2; Figure 4.35, #3) underneath although they were not distinguishable as distinct sectors from the aleurone.

A sector with a fine pattern in the aleurone indicates that there are two copies of *En* present in that sector. Therefore in the observed wx-mutable fine sector (Table 12, #3; Figure 4.35, #2) in addition to the *En* at the *wx-844* allele, there is a gain of one *En*. This type of event has previously not been detected with the a-*m1* and the *a-m(r)* reporter alleles. Thus, the use of the *a2-m1* allele has aided in detecting events that remained unobservable before. The gain of a transposable element during transposition has previously been reported with the *Ac* (*Mp*) element at the *P-vv* allele (Brink and Nilan, 1952; Van Schaik and Brink, 1959; Greenblatt and Brink, 1962, 1963; Greenblatt, 1966, 1968).

The Wx sectors provide further information on the destiny of *En*. When the Wx endosperm sectors (Table 12, #s 2,4,

and 5; Figure 4.35, #s 3,4, and 5) are considered, three types of aleurone patterns are observed. They are coarse, fine and colored. The Wx coarse sectors (Table 12, #2; Figure 4.35, #3) are a result of a simple transposition event of the *En* from the *wx-844*; excision causes Wx endosperm and the reinsertion of the *En* is detected as a coarse aleurone pattern. The Wx fine sectors (Table 12, #4; Figure 4.35, #4) indicate that *En* has excised from the *wx-844* allele and there is also a gain of an extra *En*. The colored Wx sectors (Table 12, #5; Figure 4.35, #5) are caused by the loss of *En* following excision from the *wx-844* allele.

Twin sectors were another type that allowed the determination of the destiny of *En*. In these cases, colored sectors were found juxtaposed to fine sectors (Figure 4.32). The twin occurrence of colored and fine sectors indicates their common origin. Such sectors have previously been observed in the case of the *P-vv* allele containing the *Ac* transposable element (Brink and Nilan, 1952; Van Schaik and Brink, 1959; Greenblatt and Brink, 1962). The study of twin sectors with the *P-vv* allele has provided information about the timing of the *Ac* transposition event (Van Schaik and Brink, 1959; Greenblatt and Brink, 1962, 1963; Greenblatt, 1966, 1968, 1974, 1984; Chen et al., 1987). Therefore, a systematic attempt was made to increase the frequency of twin sectors with the *wx-844* and the *a2-m1* line. This was thought to be possible because in the case of the *P-vv* study, different

frequency of twin sectors were observed on different inbred backgrounds.

The following argument was used to change the genetic background of the plants carrying the *wx-844* and the *a2-m1* alleles. The twin phenotypes would be recognizable only if they are distinct in an aleurone background of coarse spotting. The visibility of the twin sectors would also be affected by the frequency of occurrence of the events. An increased frequency of events will make the background less distinguishable from the coarse spotting pattern expected from the presence of one *En*. This will reduce the distinctness of other types of spotting patterns. Further, an increased frequency of events would also promote the occurrence of a second event following a first event. This would cause incidents of sectors within sectors and hence, will obscure the visibility of twin sectors. The observations described in Table 12 and Figure 4.35 were all on single sectors since the genetic background of the material used promoted comparatively more early and frequent excision events. In order to promote the visibility of twin sectors, the *wx-844* and the *a2-m1* alleles were transferred to an inbred background known to produce more late excision sectors and a lesser number of early excision sectors at the *wx-844* allele. It should be noted that all the earlier observations were true in the new background.

In this new inbred background, frequently occurring colored sectors were found juxtaposed to fine sectors (Figure 4.32). A representative sample of such twin sectors are shown in Figure 4.32. In these, the colored sectors were Wx and the fine sectors were wx-mutable (Figure 4.33). These give the appearance of twin sectors suggesting their origin from a single event, i.e., the same event led to the gain of one *En* in one daughter cell giving rise to the fine sector and the same event also caused a loss of the *En* the other daughter cell giving rise to the colored sector. The colored co-twin sector is Wx and therefore, the twin event is a consequence of an excision event of the *En* element from the *wx-844* allele. The event includes excision of *En* in one daughter cell while retaining an intact *wx-844* allele in the other daughter cell.

The 'loss and gain' type of twin sector was the most obvious and prevalent twin sectors observed. The easier visibility of the 'loss and gain' type of twin sectors is because the twin phenotypes would be recognizable only if they are distinct in an aleurone background of coarse spotting. This implies that only sectors having a spotting pattern other than coarse spotting would be recognizable as twin sectors. A pair of twin events will merge with the background giving the appearance of an isolated sector if one of the pair has a coarse spotting pattern.

Some isolated fine sectors were scraped to look for possible twin pairs, i.e., if it is adjacent to Wx endosperm and coarse aleurone sectors. Some of the isolated fine sectors did not reveal any adjacent sector other than the background (Figure 4.34 A). A very few isolated fine sectors showed a Wx area with coarse spotting adjacent in the aleurone and is shown in Figure 4.34B. Assuming that these adjacent occurring sectors are twin events one can describe them as 'no-loss and gain' sectors as opposed to the 'loss and gain' sectors described previously. In the 'no-loss and gain' types, one of the co-twin is Wx endosperm with coarse aleurone pattern and the other co-twin is wx-mutable endosperm with fine aleurone pattern.

The frequent occurrence of colored areas in the endosperm and their association with excision of *En* from *wx-844* suggests that the loss of *En* following excision is a more frequent event. The frequent occurrence of 'loss and gain' type of twin sectors suggests the model that transposition of *En* from a replicated part to another replicated part is of wide occurrence and explains why frequent loss events are observed during somatic excision.

Table 10. Types of sectors observed with *wx-844* and the *a-m(r)* alleles

Aleurone Sectors	Endosperm	Event
1. Spotted high	wx-m fi	Background
2. Spotted high	Wx	Transposition of *En* from *wx-844* to elsewhere (Figure 4.28 A)
3. Spotted low	wx-m fi	1. Change of *En* at *wx-844* to a lower state 2. Change of *I-102* to a lower state and still capable of suppressing (Figure 4.28 C)
4. Spotted low	wx-m co	Change of *I-102* to lower and non-suppressing state
5. Spotted low	Wx	Concomitant transposition of *En* from *wx-844* and change of state of either *wx-844* or *a-m(r)* or both (Figure 4.28 B)
6. Colored	wx-m fi	Transposition of *I-102* to elsewhere and reversion of *a-m(r)* to *A*
7. Colored	wx-m co	Loss of *I-102* after excision
8. Colored	Wx(rare)	Concomitant excision of both *En* and *I-102*
9. Colorless	wx-m fi	Transposition of *I-102* to elsewhere and reversion of *a-m(r)* to *a-nr* (Figure 4.27 B)
10. Colorless	wx-m co	Reversion of *a-m(r)* to *a-nr* and loss of *I-102*
11. Colorless	Wx	Reversion of *wx-844* to *Wx* and loss of *En* after excision (Figure 4.27 A)

Table 11. Types of somatic sectors observed with the *a-m1 5719* allele when *wx-844* is present in one dose

Aleurone	Endosperm	Event
1. Spots with pale sectors	wx-m co	Background
2. Spots with with pale sectors	Wx	*En* transposed from *wx-844*,
3. Pale	Wx	Loss of *En* after excision (Figure 4.29 B)

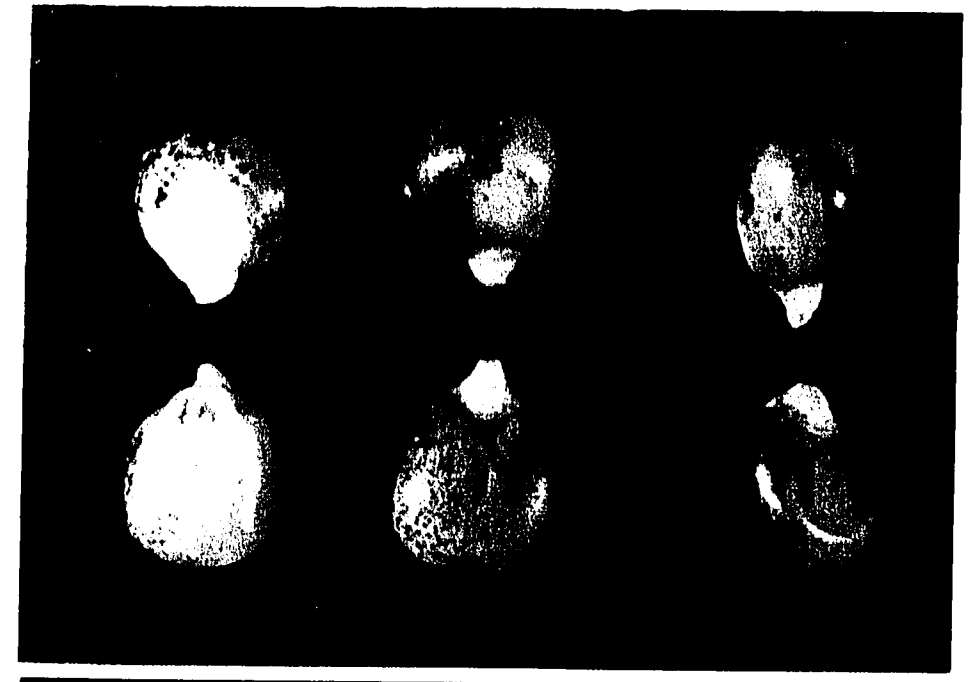
Table 12. Types of sectors observed with the *a2-m1* allele in the presence of one dose of *wx-844*

Aleurone sectors	Endosperm	Event
1. co	wx-m	Background. (Figure 4.35, #1)
2. co	Wx	Excision of *En* from *wx-844* and reinsertion elsewhere. A normal transposition event. (Figure 4.35, #3)
3. fi	wx-m	Gain of one *En* in a sector while retaining the one at *wx-844*. (Figure 4.35, #2)
4. fi	Wx	A secondary transposition event. Likely, one round of transposition results in co and Wx sector. A subsequent event similar to the above (#3) will result in a fi and Wx sector. (Figure 4.35, #4)
5. Cl	Wx	No insertion of *En* after excision from *wx-844*. A loss event following excision. (Figure 4.35, #5)

Figure 4.26 Types of sectors observed in the aleurone with the *a-m(r)* and the *wx-844* allele

A. Individual kernels (Progeny of cross: *a/a wx/wx* X *a-m(r)/a wx-844/wx* or *a/a wx/wx* X *a-m(r)/a-m(r) wx-844/wx-844*) illustrates a variety of sectors ranging from colorless to low spotted.

B. A distinct colorless sector in a background of high spotting. The genotype of the kernel is *a-m(r)/a or a-m(r) wx-844/wx*



A

B

Figure 4.27 Nature of wx mutability of the colorless sectors observed with the *a-m(r)* and the *wx-844* allele

Cross: Same as in figure 4.26

A. A colorless aleurone sector reveals a Wx endosperm sector underneath. The event illustrates a likely loss of *En* after excision from the *wx-844* allele.

B. A colorless aleurone sector is wx mutable in the endosperm illustrating a likely transposition of the *I* element from the *a-m(r)* allele.

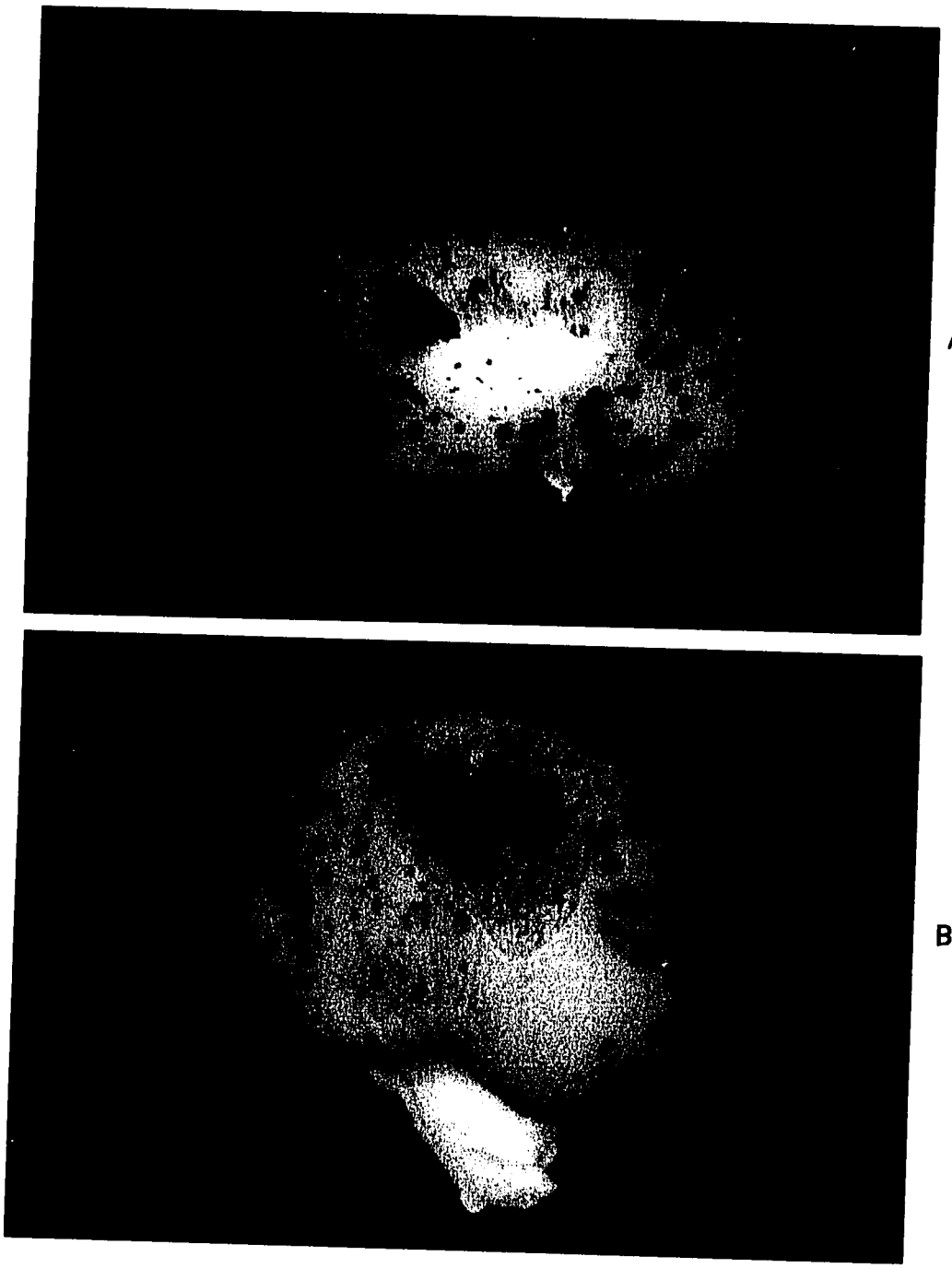

A
B

Figure 4.28 Excision events illustrating various destinies of *En*. Aleurone mutability correlated with events at the *wx-844* allele

Nature of wx mutability of the low and high spotted sectors observed with the *a-m(r)* and the *wx-844* allele

Cross: Same as in figure 4.26

A. A Wx sector which is high spotted in the endosperm. *En* has reinserted elsewhere after excision from the *wx-844* allele.

B. A low spotted sector which is Wx in the endosperm. A likely *En* change to a lower state after it has excised from the *wx-844* allele.

C. A low spotted sector which is wx-mutable in the endosperm. A likely change of the *a-m(r)* allele to a lower state and still retaining the suppressing ability

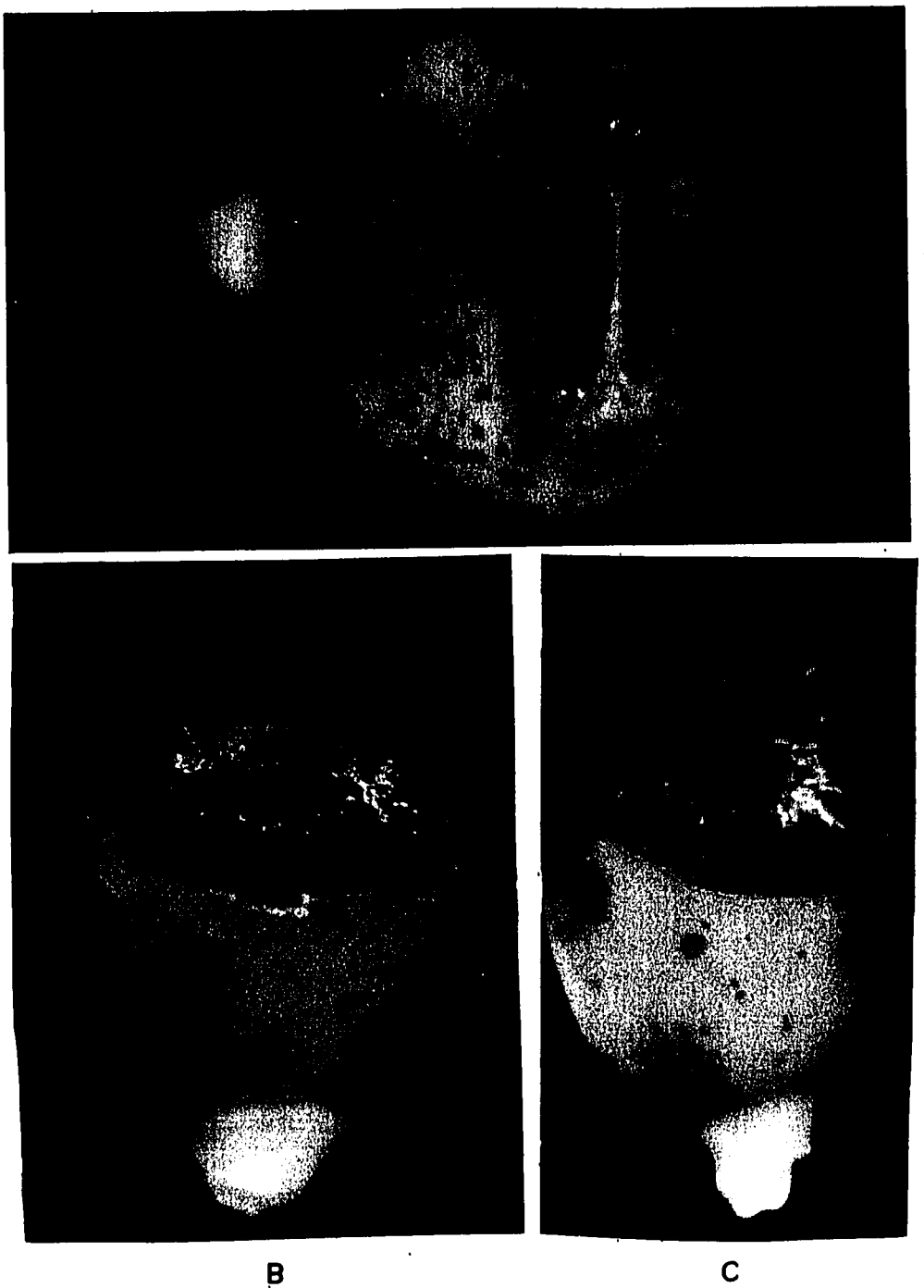

A
B
C

Figure 4.29 Use of the *a-m1 5719* reporter allele to monitor excision events of the *wx-844* allele. Association of excision with the pale sectors

Cross: *a/a wx/wx* X *a-m(r)/a-m1 wx-844/wx*

A. Phenotype of *a-m1 5719* with one dose of *wx-844*. Illustrates dark and smaller excision sectors and also various sizes of pale sectors. Some large pale sectors have colorless areas inside them.

B. Nature of the wx staining underneath the pale areas. Note that the pale sector exactly coincides with the Wx sector indicating the absence of *En* after excision.

A

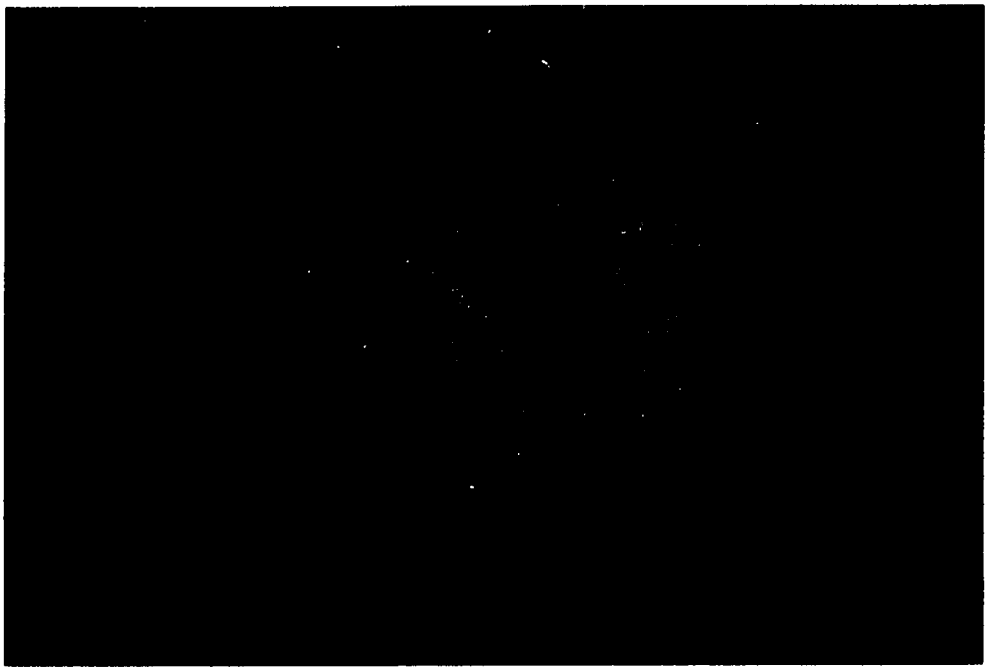
B

Figure 4.30 Nature of ringed areas of the *a-m2 8004* allele to illustrate events at the *wx-844* allele

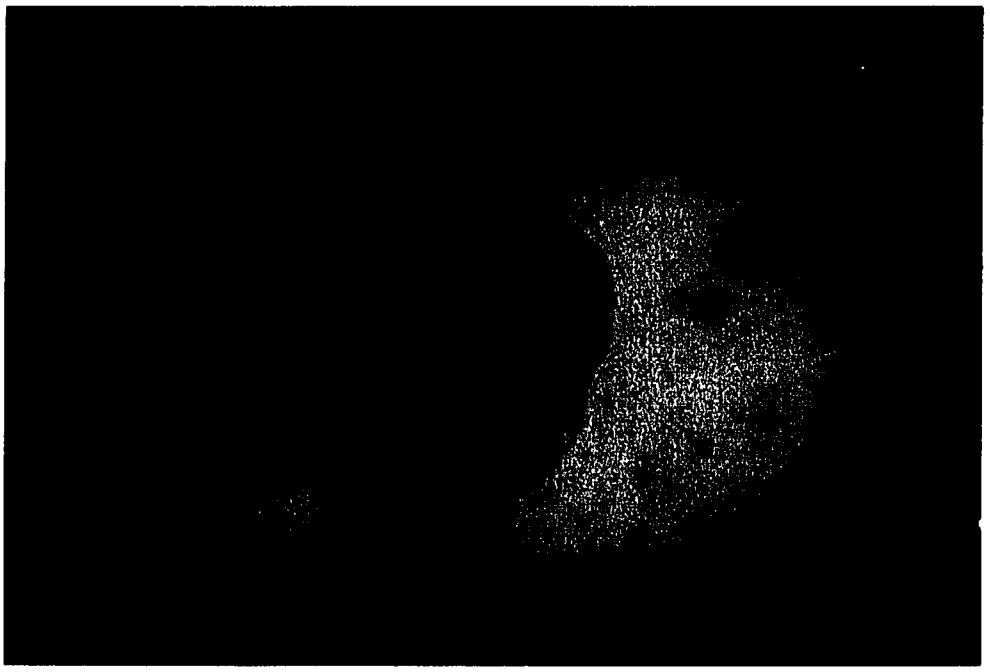
The genotype of the kernel is *a-m2 8004/a wx-844/wx*

A. A ringed area with dark border surrounding a colorless area.

B. The same ringed area scraped and stained in the endosperm. The ringed area always stains Wx.



A



B

Figure 4.31 Coarse aleurone sectoring pattern of the *a2-m1* reporter allele in the presence of one *En*

Note the closely spaced and large size of sectors. The genotype of the kernel is *a2-m1/a2-m1 wx-844/wx*

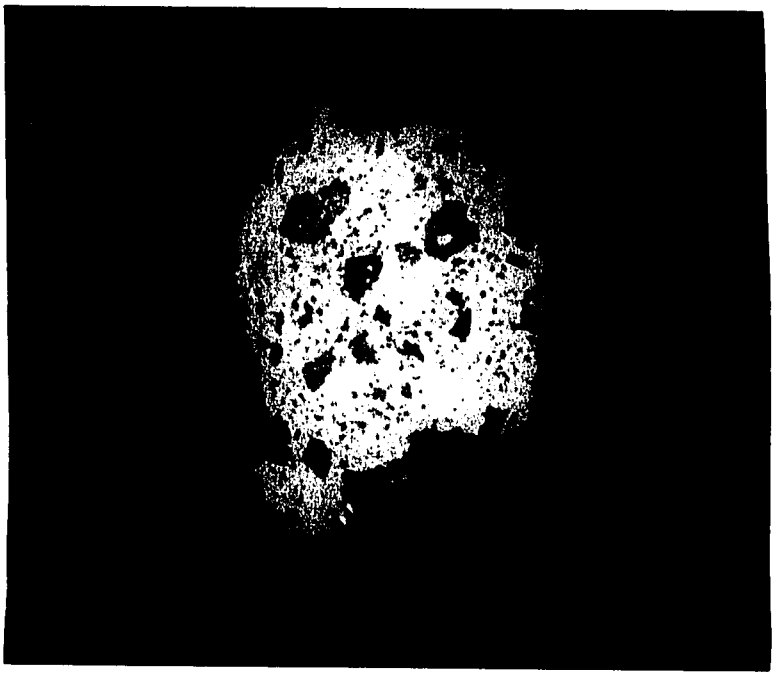


Figure 4.32 Twin sectors revealed by the *a2-m1* reporter allele in the presence of one dose of *wx-844*

The genotype of the kernels are *a2-m1/a2-m1 wx-844/wx*.

The adjacent occurrence of colored and fine pigmented areas signifies the loss of *En* in the colored area and the gain of *En* in the fine area. Only this type of twin sectoring is visible in a background of coarse spotting.

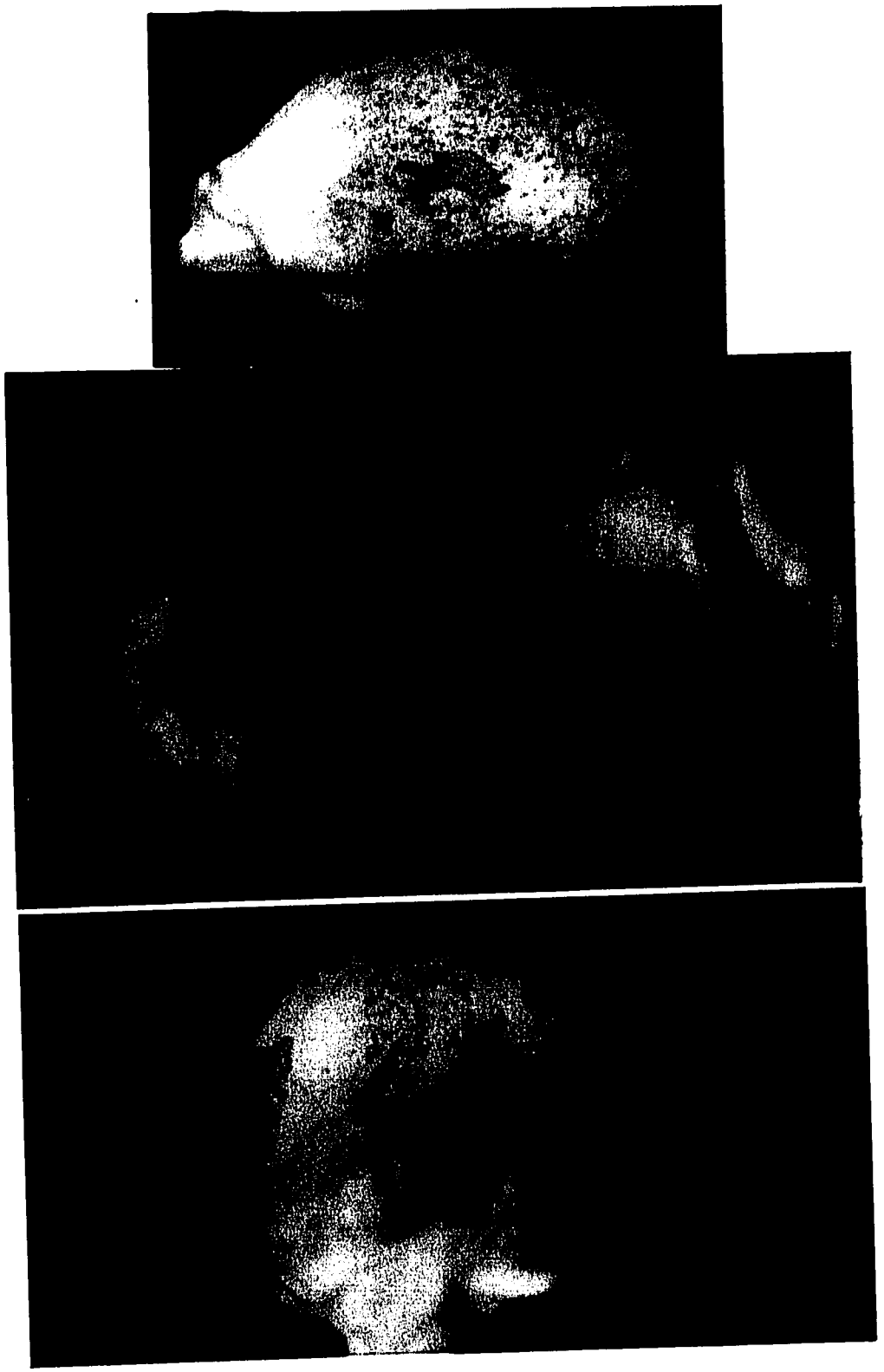


Figure 4.33 The nature of wx-mutability of the twin sectors observed with the *a2-m1* and the *wx-844* alleles

The genotype of the kernel is *a2-m1/a2-m1 wx-844/wx*.

A. The intact kernel with a twin sector: a fine sector next to a colored sector. The endosperm staining underneath this twin sector is illustrated in B.

B. The colored co-twin is Wx in the endosperm and the fine spotted co-twin is wx-mutable in the endosperm.

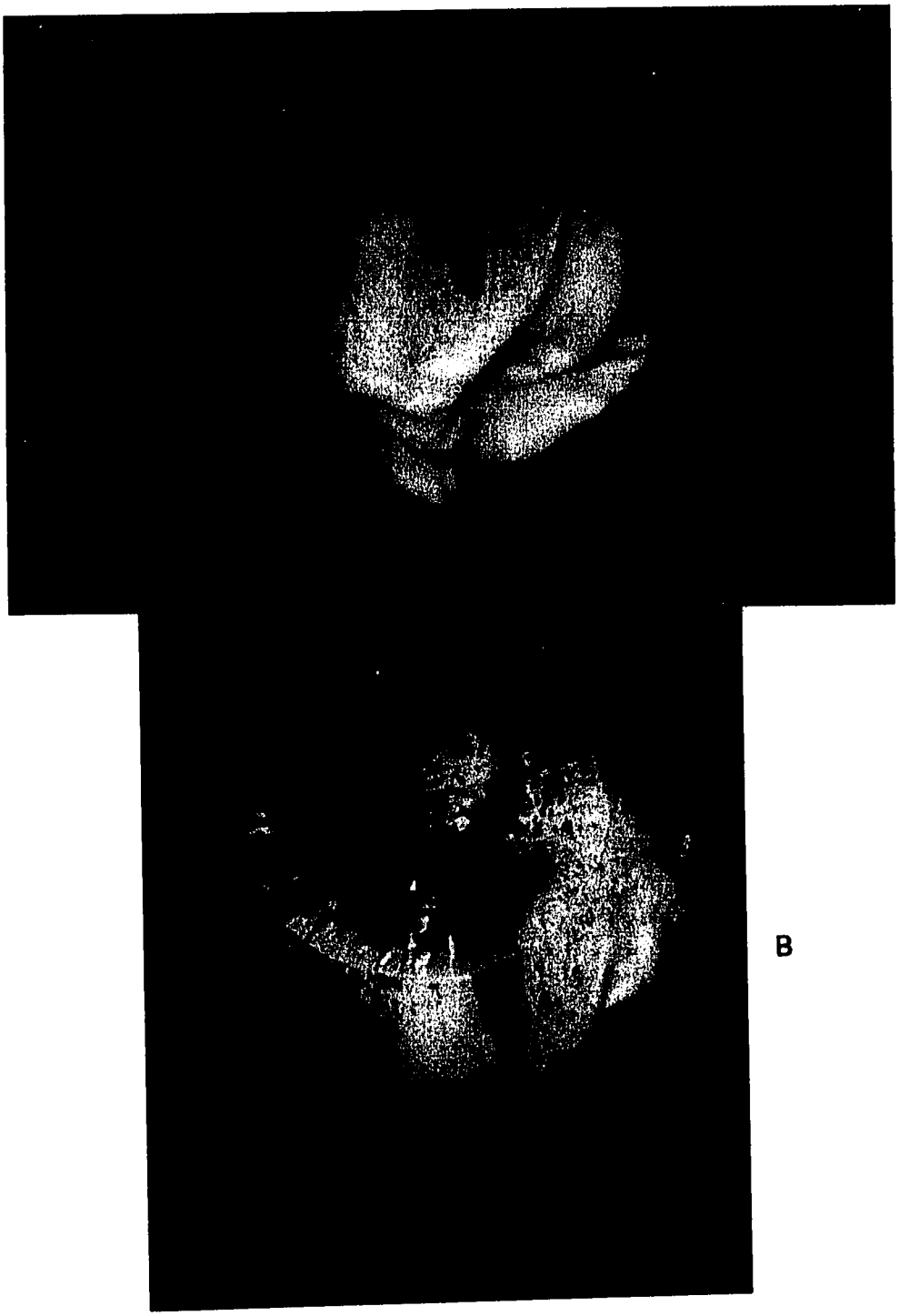
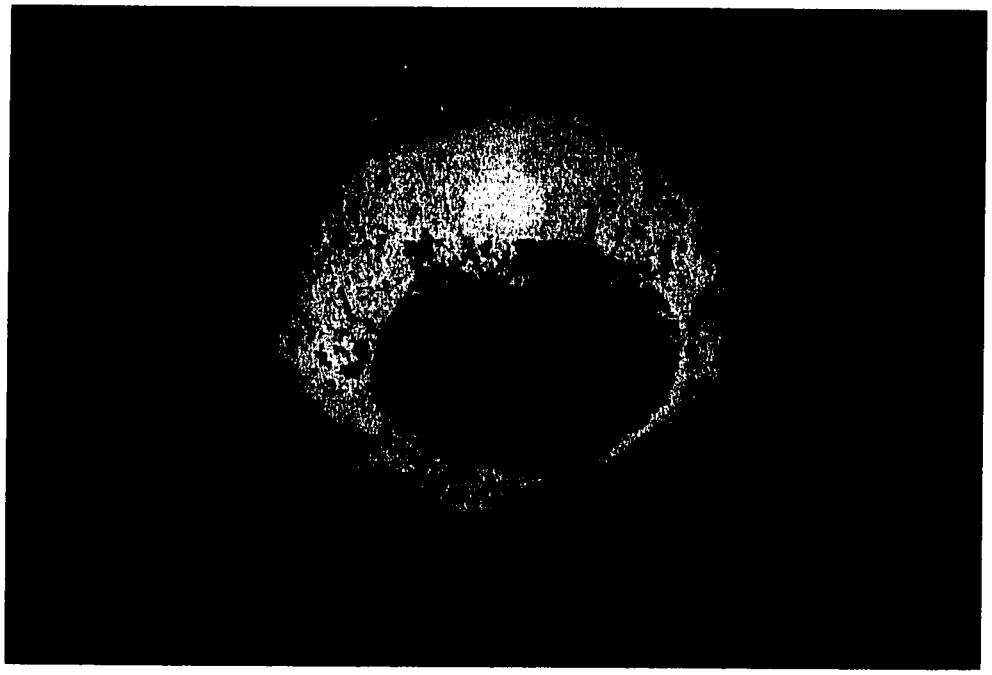

A
B

Figure 4.34 The nature of wx-mutability of the isolated fine sectors observed with the *a2-m1* and the *wx-844* alleles

The genotype of the kernels are *a2-m1/a2-m1 wx-844/wx*.

A. The isolated fine sector is wx-mutable in the endosperm and is surrounded by coarse spotted wx-mutable background.

B. The isolated fine sector is wx-mutable and is located next to a Wx sector which is coarse spotted in the aleurone.



A

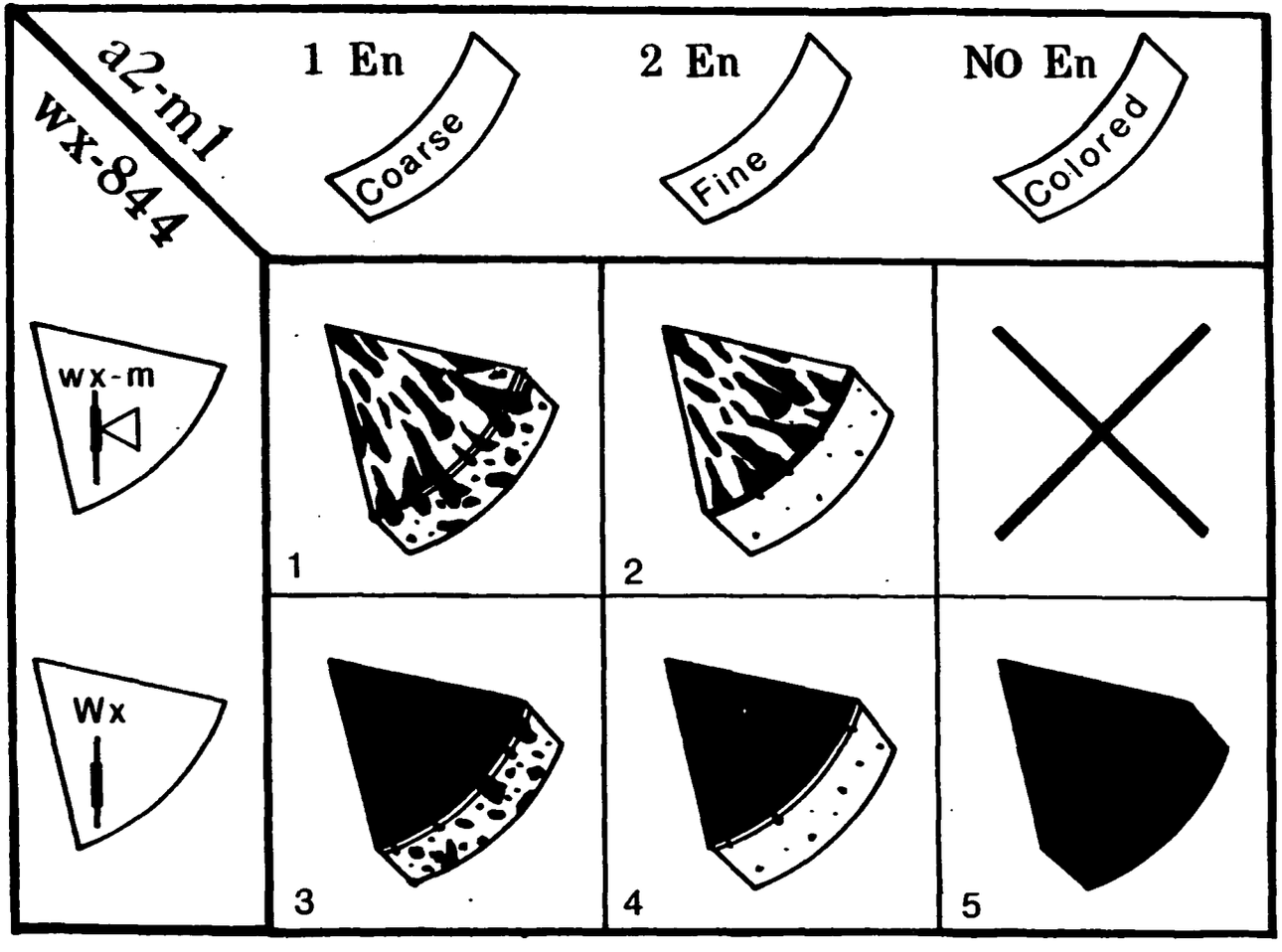
B

Figure 4.35 Diagramatic view of the nature of sectors observed with the *a2-m1* allele in the presence of one dose of *En* at the *wx-844* allele

The aleurone phenotype (*a2-m1*) is described at the top and the endosperm phenotype is described to the left of each box.

The kernels used are of the genotype *a2-m1/a2-m1 wx-844/wx* and originated from the cross: *a2-m1/a2-m1 wx/wx* X *a2-m1/a2-m1 wx-844/wx*

PHENOTYPES
wx-844::En & a2-m1::I



5. DISCUSSION

5.1. Modifying Effect of the *I-102* Element

5.1.1. Establishment of *I-102* as the modifier

5.1.1.1. Initial observation of two wx-mutable patterns

The initial observation was the segregation of two wx-mutability patterns among the progeny of the cross, *a-m(r)/a-m1 wx-844/wx* X *a/a wx/wx*. One was a coarse pattern consisting of many early wx-->Wx sectors (Figure 4.1B). The second was a fine wx-mutability pattern with reduced number and size of wx-->Wx sectors (Figure 4.1A).

The wx-mutability observed in the endosperm is due to the presence of the *wx-844* allele. From the segregation data presented in Table 1 it is clear that the wx-mutability class (columns A and B) is segregating in the ratio 1:1 with the non-wx-mutability class (columns C and D). The ratio with 1/2 wx-mutable kernels strongly suggests that only one wx-mutable allele is segregating in the cross and the two patterns must result from the modification of the expression of this allele. This wx-mutable allele is the *wx-844* allele.

Table 1 also shows that only 1/2 of the progeny (columns A and B) are spotted. This indicates that only one segregating *En* is present. This *En* is at the *wx-844* allele. This

argument obviates any consideration that two different *En*s or two different wx-mutable alleles cause the appearance of the two types of wx-mutable patterns. In summary, these observations support the contention that a single *En* containing wx-mutable allele (*wx-844*) segregates for two different wx-mutable patterns.

5.1.1.2. Coarse wx-mutability is the original pattern *wx-844* Similar observations have been reported with the *En-I* system at other mutable alleles (Peterson, 1976; Reddy and Peterson, 1983). A segregating factor *En-malt* has been shown to cause fine mutability at the *c-m* alleles (Reddy and Peterson, 1983). In the case of the autonomously mutable *c-m* alleles, coarse spotting is the basic or unmodified mutability pattern. The basic coarse pattern is changed to a fine pattern in the presence of the modifier *En-malt*. This phenomenon is similar to the appearance of coarse and fine wx-mutability patterns with the *wx-844* allele. In the case of the *wx-844* allele, one of the two patterns should be the basic pattern and the other pattern must be the modified pattern.

Coarse wx-mutable kernels have been selfed. From these selfed ears only coarse wx-mutability appears among the progeny (notes under Table 1 and Section 4.1.1.2) and thus

supporting the hypothesis that coarse wx-mutability breeds true. In contrast, among the progeny from the selfing of fine wx-mutable kernels both fine and coarse wx-mutable kernels are found to segregate among the progeny (Section 4.1.1.2 and Table 2a). This result provides proof that the coarse wx-mutability is the basic pattern of the *wx-844* allele as it does breed true. The fine wx-mutability is a modified pattern since it segregates into both patterns among the progeny.

Selfed progeny of fine wx-mutable kernels show a 3:1 ratio of coarse and fine wx-mutable kernels (Table 2a). This ratio is evidence for a single independently segregating factor that is associated with the appearance of the fine wx-mutability. This factor or the modifier is identified in the next section.

5.1.1.3. <u>The *a-m(r) 102* allele containing the *I-102* element is the modifier of coarse wx-mutability</u> The result presented in Table 1 reveals that the fine wx-mutability pattern is associated with the high aleurone spotting pattern of the *a-m(r)* reporter allele (Section 4.1.1.1) and this is characteristic of the *a-m(r) 102* allele segregating in the cross. This strict association of pattern type with the *a-m(r) 102* allele is further strengthened by segregation ratio (Table 2a and 2b, Section 4.1.1.2) and the reconsti-

tution test (Table 3, Section 4.1.1.3) provides further support for this along with additional segregation data. Removal of the *a-m(r) 102* allele by segregation causes the fine wx-mutable pattern to disappear and the reintroduction of the *a-m(r) 102* allele causes the fine pattern to reappear. This is a proof that the *a-m(r) 102* allele is the modifier of coarse wx-mutability. Effect of any other segregating factor has been ruled out by sib crossing among the segregating progeny (Section 4.1.1.3).

The *a-m(r) 102* allele is an *I* element inserted within the *A* gene (Schwarz-Sommer et al., 1987). Therefore this *I* element, the *I-102*, is the modifier. Whether associated linkage with this element could be affecting this pattern is challenged by the transposed *I-102* element.

A transposition of the *I-102* element away from the *A* locus has also been obtained (Section 4.1.2). The *I-102* element transposed to a locus independently segregating from the *A* locus. The transposed *I-102* element is also shown to similarly repress the coarse wx-mutability. This observation further supports the modifying role of *I-102* and eliminates the possibility that a factor linked to the *a-m(r) 102* allele acts like a modifier. If a linked factor were responsible, one should not expect the transposed *I-102* to affect the coarse wx-mutable pattern. These results conclusively prove that the resident *I-102* element of the *a-m(r) 102* allele is

the modifier or the repressor of the coarse wx-mutability to a fine pattern.

5.1.1.4. *I-102* also represses other mutable alleles

The generality of the reducing effect of *I-102* on *wx-844* mutability has also been tested at other mutable alleles. They include one autonomously mutable allele and five non-autonomously mutable alleles.

The test of repression of the *a-m(Au)* (Section 4.1.3) and the *a-m1 6078* (Section 4.1.4) alleles was possible after isolating a transposed *I-102* element (Section 4.1.2). The *a/a* genotype carrying the transposed *I-102* was used to observe the repression at the a-mutable alleles after verifying the repressing ability of the transposed *I-102* in Section 4.1.2. The *a/a* genotype with the independently segregating *I-102* element facilitated observation of mutability of the *a-m(Au)* and the *a-m1 6078* alleles without the interference from the mutability of the *a-m(r)* allele. Both the *a-m(Au)* (Section 4.1.3) and *a-m1 6078* (Section 4.1.4) alleles respond to the repressor effect of the *I-102* element and their mutability is repressed from a coarse sectored pattern (Figures 4.2B left, 4.3A bottom) to a significantly reduced fine (6-7b) pattern (Figures 4.2B right, 4.3A top).

The *a-m(r) 102* allele was used to detect its reducing effect at the *c-m(r)* (Section 4.1.7) and the *c2-m2* (Section

4.1.8) alleles. A strong correlation between the presence of the *a-m(r)* allele and the reduction of mutability at both the *c-m(r)* (Section 4.1.7; Flow diagram 4) and the *c2-m2* (Section 4.1.8; Flow diagram 5) alleles has been established.

A similar reduction of mutability has also been shown for the *a-m1 5719* (Section 4.1.5) and the *a-m2 4412* alleles (Section 4.1.6). The *a/a* genotype containing the transposed *I-102* element (Section 4.1.2) was also used to observe the reduction of mutability at these two alleles. Again, in this way the interference from the mutability at the *a-m(r)* allele was eliminated. These observations suggest that the reducing effect of the *I-102* element is a general phenomenon for all *En* mediated mutability.

5.1.2. The molecular structure and expression of *I-102*

5.1.2.1. Molecular structure of *I-102* reflects putative product forming capability

The molecular structure of the *I* element of the *a-m(r) 102* allele has been determined by Schwarz-Sommer et al., 1987. After the detection of the reducing effect of the *a-m(r) 102* allele, a possible product forming capacity of this allele was sought from the deletion points reported by Schwarz-Sommer et al., 1987.

This *I* element is 3.7 kb long and the end points of its deletion were found by Schwarz-Sommer et al. (1987) to be

located at positions 1840 and 6430 of the *En1* element (Pereira et al., 1986). Based on the deletion points and comparing this to the sequence of *En1*, this *I* element retains the highly structured termini of the *En* structure. The left end of this *I* element retains the *En* promotor (Figure 5.1), the first exon of tnpA and a part of the ORF1. At the right end, exons 7 to the end of the element remain unaffected (Figure 5.1).

On the basis of the deletion end points provided by Schwarz-Sommer et al. (1987) the potential ability of this *I* element to form a product was speculated and is described as follows. From this *I* element structure, a primary transcript of a shorter length than that of *En* can be obtained starting from the promotor at the left end of this element (Figure 5.1) and proceeding to the end of the element. Processing of the primary transcript may include exon 1 and exons 7 to 11 of tnpA (Figure 5.1). Alternatively, the processed mRNA may also include a part of the ORF1. Inclusion of a part of the ORF1 is not unlikely since there are potential splice donor and acceptor sites within the ORF1 (Pereira et al., 1986). The overall reading frame may be maintained to be able to form a translation product. Although the normal translation start site of tnpA (Figure 5.1) is eliminated in the deletion, an ATG triplet is present at the beginning of the ORF1 and is not included in the deletion (Figure 5.1). With these

features still present, it is not unlikely for a potential product to be formed by this *I* element. This product may be involved in the reducing effect caused by the *I-102* element.

To test the just described argument, the *I-102* element from the *a-m(r) 102* allele showing the modifying effect on wx-mutability was cloned by Cuypers et al. (1988). This was necessary because the *I-102* element characterized by Schwarz-Sommer et al. (1987) was not tested for its modifying effect of *En* mediated mutability.

This confirmed *I-102* element (Cuypers et al., 1988) is 3697 bp in length and is derived from the *En1* sequence by an internal deletion of 4590 bp removing nucleotides 1862-6451 Pereira et al., 1986) (Figure 5.1). The structural features described earlier for the *I* element cloned by Schwarz-Sommer et al. (1987) remain valid for the *I-102* element cloned by Cuypers et al. (1988). Hereafter in the discussion the *I-102* element or the *a-m(r) 102* allele mentioned would refer to the *a-m(r) 102* allele and the resident *I-102* element described and characterized by Cuypers et al. (1988) and is shown in Figure 5.1.

5.1.2.2. Transcription and gene structure of the *I-102* element: tnpR as the gene product The product forming ability of the *I-102* element has been discussed in the previous section. Because of this reason, the *a-m(r) 102* allele,

showing the modifying effect on wx-mutability was cloned and characterized at the Max Planck Institute (Köln, Germany) by Cuypers et al. (1988). Three transcripts have been detected (Cuypers et al., 1988) which appear to be specific for the *a-m(r) 102* allele. Such transcripts have not been observed within the *wx-844* line, when the *a-m(r) 102* allele is segregated out. The most abundant *a-m(r) 102*-specific transcript is 1.8 kb and the two other minor transcripts are 2.3 kb and 3.0 kb in size.

The same pattern of transcripts are also found in lines carrying the transposed *I-102* element. The RNA pattern also co-segregates with the plants carrying transposed *I-102* among the progeny segregating for the transposed *I-102* element detected by wx-mutability test (Cuypers et al., 1988). This is substantive confirmatory evidence for the hypothesis that the *I-102* element is the modifier that alters the pattern of the *wx-844* allele from that of coarse mutability to fine mutability.

The cDNA corresponding to the most abundant 1.8 kb transcript has been identified and characterized (Cuypers et al., 1988). It contains seven exons and spans almost the entire length of the element (Figure 5.1). The gene structure is very similar to the one that was predicted in the previous section. Transcription is initiated from the promotor at the left end of the element. The *I-102* cDNA contains exon 1 of

tnpA, part of the ORF1 which becomes the second exon, and exons 7-11 of tnpA which form the last five exons (Figure 5.1). The first ATG codon is at the beginning of the second exon (Figure 5.1) and the predicted size of the protein product would be 386 amino acids. A protein of corresponding size, 40 kd has been observed when the cDNA was expressed in an in vitro system (Cuypers et al., 1988). This gene has been identified as tnpR (R for reducer of mutability).

When compared to the tnpA structure of *En1* (Figure 5.1), the tnpR shares with tnpA the first exon as well as the last five 3'-terminal exons. The difference is the incorporation of the ORF sequences in tnpR and the elimination in tnpR of exons 2 through 6 of tnpA. Therefore tnpA and tnpR encode putative proteins with unique amino-terminal domains and identical carboxy-terminal domains (exons 7-11 of tnpA and the last five exons of *I-102* as shown in Figure 5.1 and mentioned in the legend).

5.1.3. Mode of action of *I-102* mediated reduction of excision

5.1.3.1. *I-102* reduces the number of excisions and delays the events In the absence of the *I-102* element the *wx-844* allele shows a coarse pattern of wx-mutability. The coarse pattern consists predominantly of larger wx-->Wx sec-

tors and the wx-->Wx sectors are also more frequent. Presence of the *I-102* element causes the *wx-844* allele to express a fine pattern of wx-mutability. The fine pattern consists of smaller and fewer wx-->Wx sectors. The wx-->Wx sectors are due to the excision of the *En* at the *wx-844* allele. Larger wx-->Wx sectors indicate that the excision event has occurred earlier during the development of the endosperm. Smaller wx-->Wx sectors indicate that the excision event has occurred later.

Therefore, a change from predominantly coarse to fine pattern of wx-mutability indicates a delay in the excision events. A change from a higher number of wx-->Wx sectors to fewer such sectors indicates that the number of excision events is reduced and delayed. Therefore *I-102* mediated modification of a coarse pattern to a fine pattern is essentially a reduction in the frequency of excisions and delay in the timing of the excision events during endosperm development. The same explanation is true for suppression of mutability observed at other *En* related mutable alleles.

5.1.3.2. <u>The *I-102* does not affect the S function of *En*: only the M function of *En* is affected</u> The interaction between the *I-102* element and the *a-m1 5719* reporter allele provides an ideal instance of the effect of the *I-102* element on the S function of *En*. In the presence of *I-102*,

the *a-m1 5719* allele shows a lesser number of spots, i.e., the number of excision events are reduced compared to what is expected. But the suppression of the pale background color of the *a-m1 5719* allele remains unaffected in the presence of the *I-102* element (Section 4.1.5). This is a strong indication that the S function of *En* is not influenced by the *I-102* element.

The *En* mediated suppression of the background color of the *c2-m2* allele is also not affected by the presence of the *a-m(r) 102* allele (Section 4.1.8). In this case, only the number and size of *En* mediated c2-->C2 excision sectors are reduced (Section 4.1.8; Figure 4.10). Similarly, a lack of influence of the *I-102* element on the coex function of *En* has been observed in the case of the *a-m2 4412* allele (Section 4.1.6). The coex function is essentially an equivalent of the S function of *En* (McClintock, 1971).

The conclusion can be drawn that the *I-102* element only affects the *M* function of *En* and has no detectable effect on the expression of the *S* function *En*. The tnpA product has been shown to be the DNA binding protein responsible for The S function of *En* (Gierl et al., 1988b). The S function is expressed by virtue of the binding of the tnpA product to the ends of the element (Gierl et al., 1988a, 1988b; Grant et al., 1990) and prevents read-through transcription. Since the S function of *En* is not affected, it follows that the

functioning and expression of tnpA is not seriously hampered by tnpR. This was confirmed by Cuypers et al., 1988 when they demonstrated that the level of the 2.5 kb *En* encoded transcript for tnpA remains the same irrespective of the presence of the *I-102* element in northern experiments. No effect on the amount of the 2.5 kb transcript would imply that the promotor at the left end of *En* is probably not the target of tnpR.

5.1.3.3. <u>The tnpR interaction is likely at the protein level</u> The tnpR expresses part of the ORF1. A possibility exists that the expression of the *En* transcript(s) that expresses the ORFs might be reduced in the presence of tnpR. Since the level of these transcripts are already very low, it has been difficult to verify this possibility (Cuypers et al., 1988). The tnpR may be considered to be composed of two domains, the amino-terminal domain containing part of the ORF1 and the carboxy-terminal domain containing the last five exons. These last five exons are identical to exons 7-11 of tnp A. Thus, tnpR shares the carboxy-terminal domain with tnpA but both have unique amino-terminal domains. Therefore, it was postulated by Cuypers et al. (1988) that the tnpR product might interfere with the action of the tnpA or other *En* product(s) (e.g., the product formed by the 6 kb transcript) containing identical carboxy-terminal domains. This

is more likely if the *En* encoded proteins act in a dimeric or polymeric fashion which is not unlikely.

The action of the *I-102* element via tnpR can be considered as an action of a partially dominant negative mutation. Such an example exists for the *tet* repressor of Tn10 (Issacson and Bertrand, 1985).

The dosage effect of the *a-m(r) 102* allele described in section 4.1.10 indicates a nearly linear relationship between the amount of reduction in excision observed and the ratio of the number of copies of *I-102* to the number of copies of *wx-844* present. If the ratio is large, the frequency of excision is relatively less than when the ratio is small. This observation agrees with a product level competitive inhibition model of *I-102* action.

5.1.4. Effect on its own excision

The *a-m(r) 102* allele shows a 7-8b spotting pattern in the presence of *En* (Figure 4.1A). This spotting pattern must reflect the effect of the tnpR produced by the resident *I-102* element on the excision of the I element from the *a-m(r)* allele. This implies that the spotting pattern reflects an already reduced pattern. The true spotting pattern without the tnpR effect would certainly be higher than what is observed.

The *a-m1 6078* and the *a-m(Au)* alleles show a spotting pattern very similar to that of the *a-m(r)* allele after the reducing effect of the *I-102* element has occurred. In other words, their original spotting pattern would also be the original spotting pattern of the *a-m(r)* allele. Therefore we predict that in the absence of the reducing effect, the *a-m(r)* allele would show a spotting pattern that would be described as coarse sectored, i.e., very similar to that of the *a-m1 6078* and the *a-m(Au)* alleles.

The prediction that *a-m(r) 102* would show a higher spotting pattern in the absence of tnpR effect corroborates very well with the structure of the *I-102* element. This *I* element has an intact 3' end termini, the likely substrate for the transposase responsible for excision. *I* elements with intact termini structures are known to show higher excision frequency, for example, the *wx-m8*, *bz-m 13*, and the *a-m1 6078* alleles (Schiefelbein et al., 1985, 1988; Tacke et al., 1986). Because the *I-102* element has intact termini structure, therefore, it should be a good substrate for excision.

From the characteristics of the *I-102* element and the *a-m(r) 102* allele studied in this dissertation, it is possible to make a few predictions on the nature of the derivatives that can be derived from the *a-m(r) 102* allele. It should be possible to isolate a derivative from the *a-m(r)* allele that

shows a spotting pattern similar to that shown by the *a-m1 6078* and the *a-m(Au)* alleles. This pattern of spotting is the predicted pattern of the *a-m(r) 102* allele in the absence of the self imposed tnpR effect. Such a predicted derivative would lack the suppressing ability of the parental *a-m(r) 102* allele. The defect in the new *I* element would be a lesion in the region that is responsible for the suppressing ability of the *I-102* element, i.e., a functional tnpR like product would not be produced. The lesion may either be in the ORF1 region or in any other region that does not affect its ability to serve as a good substrate. As long as the lesion does not affect the termini structure necessary for proper excision the derivative would show a coarse sectored pattern. By a similar argument, derivatives can be obtained that show a lower spotting pattern and still retain the ability to suppress *En* mediated excision. In this case the lesion would include a region that affect its ability to serve as a good substrate for excision but does not hamper the regions necessary for the production of tnpR or tnpR like products capable of modifying *En* mediated excision. A third possible derivative would be an *I* element that is defective in producing a functional tnpR as well as in regions that make the *I-102* element a good substrate for excision. Such a derivative would show a lower spotting pattern and in addition, would lack an ability to suppress *En* mediated excision.

5.1.5. Reduction and delay in excision

The effects of *I-102* are observed as a reduction in the frequency of excision as well as a delay in the excision event during the endosperm development. If excision frequency alone is affected, the result would be the appearance of both small and large sectors but only fewer in numbers. But the actual observation is predominantly small sectors. This implies that early during endosperm development the excision is impaired. One likely reason may be that the production of tnpR is reduced during later periods of endosperm development and hence, excision frequency of late sectors is not affected. Alternatively, appearance of delayed excision sectors could be a consequence of saturation of tnpR by excess of *En* encoded tnpA early during endosperm development.

5.2. The Low Acting *En* at *wx-m 86246X*

5.2.1. Confirmation of *En* change at *wx-844*

The *wx-m 86246X* allele was derived from the *wx-844* allele as an exceptional kernel showing a lower spotting pattern than expected from the *wx-844* allele (Section 4.2.1). The exceptional kernel was derived from the cross, *a-m(r)/a-m(r) wx/wx* X *a-m1/a-m1 wx-844/Wx*. A low spotted exceptional kernel from such a cross would imply that a change has

occurred either in the *En* element at the *wx-844* allele or at one of the reporter alleles. The likelihood of a change at one of the reporter alleles is immediately ruled out from the nature of the cross. The progeny, including the exceptional kernel from the above cross, contain the *a-m(r)* reporter allele from the tester parent. It is highly unlikely that this reporter allele would have changed among any of the progeny since it was excluded from the action of an element. The low spotting pattern (1b) observed in the exceptional kernel is much lower than that expected from either the *a-m(r)* or the *a-m1* allele. When the exceptional kernel was test crossed to the *a-m1* tester, only low spotted kernels appeared among the progeny (Section 4.2.1, Table 6A). This result confirmed that the low spotting pattern (Figure 4.15) is heritable and also confirmed that the change is not at the reporter allele since in this test cross the unchanged *a-m1* reporter allele originated from the tester parent.

Test cross results (Section 4.2.1, Table 6) also revealed that only one *En* is segregating in the this low spotted genotype. The low spotted genotype also showed reduced wx mutability (Figure 4.15) when compared to that of the *wx-844* allele indicating that a change has occurred at the *wx-844* allele. These observations collectively suggest that the *En* element of the *wx-844* allele has suffered a change to a lower state in the origin of the *wx-m 86246X* allele. The

reduced pattern in both alleles, that is spotting and wx mutability (Figure 4.15) indicates that the *En* in this *wx-m 86246X* allele is weak in its M action.

5.2.2. Molecular structure and transcription

The weak *En* element from the *wx-m 86246X* allele has been cloned by Gierl et al., 1988b and is found to be a deletion derivative of *En1*. This weak *En* element at the *wx-m 86246X* allele is identified as *En2* and bears a 1126 bp internal deletion. Compared to the *En* structure, *En2* retains (Figure 5.2) the complete sequence coding for the *tnpA* gene. The deletion affects all of ORF2 and 500 bp from the right end of ORF1. The part of the ORF1 that has significant homology with the Tam1 element (Sommer et al., 1988) is retained (Figure 5.2). From the structure, it is implied that *En2* has the potential to form the *tnpA* gene product. Further, *En2* retains that part of the ORF sequence that has been implied to have a role in the transposition process.

A weak element, *Spm-w* described by McClintock (1963) has a very similar structure (Masson et al., 1987). This weak element also retains the sequences necessary for the expression of the *tnpA* gene. *Spm-w* also shows a reduced excision rate at the *a-m2* allele as well as reduced *M* function observed with other reporter alleles (McClintock, 1963). Both *En2* (Gierl et al., 1988b) and *Spm-w* (Mason et al., 1987)

produce tnpA although at a five fold reduced rate. The ability to produce tnpA explains the observation that *En2* still retains its S function since tnpA has been shown to be the DNA binding protein necessary for the S function of *En* (Gierl et al., 1988a; Grant et al., 1990).

5.2.3. The low M action of *wx-m 86246X*

This mutant of the *wx-844* allele still retains some M action of *En* though at a significantly reduced rate. The weak M action is observed both on its own excision (wx-->Wx) as well as on the excision of other reporter alleles (Figure 4.15). The lower wx mutability observed is not due to *En2* being a poor substrate for excision. There are several observations that support this contention. First, the reduced excision is also observed at other reporter alleles, e.g., *a-m1* and the *a-m(r)* alleles and the *a-m(r)* allele is known to be a good substrate for excision since it shows a high rate of excision with a standard *En*. Since the *wx-m 86246X* allele still induces a lower rate of excision on *a-m(r)*, therefore, the lower M action is due to a weaker trans-active signal than any cis-acting defects.

Additional support is derived from the molecular structure because the ends of *En2* are intact (Figure 5.2) and the ends of the elements are known to be the substrates for excision.

The *wx-m 86246X* allele has a weaker S action when presnt in one dose (Figures 4.16, 4.17, 4.18A) as compared to the S action of the *wx-844* allele. In support of this, the tnpA transcript is also expressed at a lower level than in the genotypes carrying the *wx-844* allele (Gierl et al., 1988a). McClintock has observed that the S function is necessary for the M action. The reduced S action of *wx-m 86246X* therefore might be responsible for the weak M action observed.

Gierl et al. (1988a) have suggested that *En2* completely lacks the M function. Yet, the spots observed have been hypothesized to be caused by the activation of inactive elements present in the genome.

A weak element is potentially capable of activating inactive elements because their S function is still operative. The S function is necessary for the activation of inactive elements (McClintock, 1965b). The Spm-w element has been shown to acivate cryptic elements in the genome both transiently as well as permanently (Masson et al., 1987). Therefore the excision observed with the *wx-m 86246X* allele may be due to transient expression of cryptic elements present in the genome. These cryptic elements in turn may possess the M action and therefore are capable of excision of both *En2* from the *wx-m 86246X* allele and *I* elements of the reporter alleles like *a-m1* and *a-m(r)*.

Although the S function of *En* has been identified as tnpA, the *En* product for the for the M function has not been specifically identified. But it is speculated that the *En* specific 6.0 kb transcript expressing the ORFs of *En* might code for the putative transposase necessary for the expression of the M function. Various supports for this hypothesis has been discussed in Section 2.4.4.2. The *En*2 element, although it does not contain the complete ORF sequences, does have a major part of ORF1. The part of ORF1 that shares homology with Tam1 element is retained in the structure of *En*2. Therefore, it is not unlikely that a transcript might be formed from *En*2 that expresses the ORF1 sequences. Such a product still might be responssible for a weak excision potency. Only the detection of an *En*2 specific transcript hybridizing to ORF1 probes will help resolve the possibility.

5.2.4. The low S activity of *wx-m 86246X*

The S activity of *wx-m 86246X* has been observed with both the *a-m1* (Section 4.2.2; Figures 4.16-4.18A) and the *a2-m1* (Section 4.2.3; Figure 4.19) alleles. The *a-m1* reporter allele responds both to the M and the S functions of *En* and the a2-m1 allele responds only to the S function of *En*. The expression of the background aleurone color of the *a-m1* allele is a good indicator of the strength of the S function. The *wx-m 86246X* allele when derived from the female parent

shows a complete S potency with the *a-m1* reporter. In this case few spots are seen on a distinctly colorless background. Similarly, with the *a2-m1* reporter the background color is effectively suppressed when the *wx-m 86246X* allele is derived from the female parent. Therefore, the resident weak *En* of the *wx-m 86246X* allele shows a complete Suppressor potency when present in two doses.

The suppressor function is not fully expressed when the *wx-m 86246X* allele is derived from the male parent and thus, at one dose. This has been observed with both the *a-m1* (Section 4.2.2) and the *a2-m1* (Section 4.2.3) alleles. The incomplete S function is expressed in the form of pale to colored pigmented patches predominantly in the gown region of the aleurone when observed with the *a-m1* allele. The *a2-m1* reporter also shows a similar pigmentation pattern in the presence of the *wx-m 86246X* allele when derived from the male parent. The irregular distribution of the background color in the case of the *a-m1* and the *a2-m1* allleles is an indication of the weak or unstable S expression when the weak *En* is present in one dose, i.e., when the *wx-m 86246X* allele is derived from the male parent.

The tnpA product is responsible for expression of the S function of *En* (Gierl et al., 1988a; Grant et al., 1990). *En2*, the resident element of the *wx-m 86246X* allele produces tnpA at a five fold reduced level than a standard *En* (Gierl

et al., 1988b). The reduced level of tnpA explains the weak S action of the *wx-m 86246X* allele.

Multiple copies of *En* homologous sequences exist in the genome (Schwarz-Sommer et al., 1984; Pereira et al., 1985). Deletion derivatives of *En* similar to *I* elements constitute a major part of it. These sequences provide binding sites for the tnpA. As a consequence, the meagre amount of tnpA produced by *En2* is filtered out by these *En* homologous sequences. This results in an insufficient amount of tnpA available for binding at the *a-m1* or the *a2-m1* alleles where the direct observations are made. A lack of binding allows the transcription machinery to transcribe the coding sequence of the reporter alleles, thus yielding pigmented cells in the aleurone. Two doses of *En2*, likely, produce more than the threshold amount of tnpA needed for complete binding at the reporter alleles and hence, effective suppression of the background color is observed. The low level of tnpA production explains the dosage effect observed with the S expression of the *wx-m 86246X* allele.

5.2.5. Similarity between *wx-m 86246X* and *En*-crown

The phenotypes of *En*-crown have been described by Peterson (1966). This state of *En* is active in the crown (top of the maize kernel) of the kernel and is inactive in the gown (area at the bottom region of the maize kernel) region. As a

result, in the presence of a reporter allele, spots are observed in the crown region. This *En*-crown pattern is very similar to the background pigmentation pattern observed with the *a-m1* (Figure 4.17) and the *a2-m1* (Figure 4.19) alleles in the presence of one dose of the *wx-m 86246X* allele. In the case of the *wx-m 86246X* allele, partial inactivation of the S function is observed in the gown region and full S expression is seen on the crown region.

The *En*-crown pattern has been ascribed to the tissue specific expression of *En* activity; the *En* functions are expressed in the crown tissue of the kernel and remain un-expressed in the gown tissue. In the previous section (5.2.4), the unstable S expression of the *wx-m 86246X* allele has been ascribed to the production of insufficient amount of tnpA. Insufficiency of tnpA alone does not explain why a lack of the binding protein should be limited to the gown tissue?

Partial inactivation of *En2* in the gown region may be an alternative explanation for the gown expression of the reporter allele. Regulation of the activation and the inactivation process of *En* has also been ascribed to the binding of the tnpA protein (Gierl et al., 1989). Binding of tnpA prevents methylation of CG residues at the binding sites. A reduced level of tnpA produced by *En2* predisposes the methylation sensitive sites to methylating enzymes leading to

inactivation of the element. It is possible that the tnpA production is more adversely affected in the gown tissue of the kernel than in the crown tissue. Tissue specific inactivation is known to occur in the *En/Spm* system (Fedoroff and Banks, 1988).

The cause of tissue specific inactivation may be due to the differential level of expression of tnpA in gown and the crown regions. The production of tnpA is dependent on splicing of the primary transcript. Tissue specificity of splicing of the transcript is known in the *Drosophila P* transposable element (Laski et al., 1986). In the case of *En*, splicing of the primary transcript is probably the cause of differential level of expression of tnpA and tnpD in maize and tobacco. For example, tnpA is more abundant in maize whereas tnpD is more abundant in tobacco (Pereira and Saedler, 1989). Therefore it is not unlikely that in a defective element like *En*2 the regulation of expression of the tnpA is affected by splicing. The irregular level of tnpA availability thus would affect the transcription blockage caused by the binding of the tnpA to regulatory sequences at the left end of the element.

5.2.6. Leaky expression of the Wx gene in the *wx-m 86246X* allele

With one dose of the *wx-m 86246X* allele, the endosperm staining shows a background Wx expression in addition to the wx-->Wx sectors (Figure 4.23) caused by excision of *En2*. This background expression is observed throughout the endosperm tissue and the intensity of the background is affected by the dosage of the *wx-m 86246X* allele present. In genotypes carrying two or more doses of *wx-m 86246X* the background Wx expression varies from none to a background of minimal intensity. In contrast, in genotypes carrying one dose of the *wx-m 86246X* allele, a stronger Wx background expression is observed (Section 4.2.5; Figures 4.22-4.24).

This observation is very similar to the dosage effect of the *wx-m 86246X* allele on the background aleurone pigmentation when the *a-m1* and the *a2-m1* reporter alleles are used. In both cases, stronger background expression is observed when one dose of the *wx-m 86246X* allele is present compared to minimal background gene expression when two or more doses of the *wx-m 86246X* allele are present.

This background expression may be ascribed to the varied suppressibility of the *wx-m 86246X* similar to that of the *a-m1* and the *a2-m1* alleles. With *wx-m 86246X*, the transcription from the Wx gene continues into the *En2* sequence and into the remaining portion of the *Wx* gene and this is fol-

lowed by splicing of the insert sequence in the mature mRNA. In the presence of one dose of *En2*, the tnpA level is not sufficient to occupy all the insert binding sites and hence some transcription occurs at the *Wx* gene. Two doses of *En2* produce enough tnpA to occupy the insert binding sites and prevent transcription from the Wx gene into the *En2* sequences.

An interesting observation is that the Wx background expression is prevalent through out the endosperm tissue whereas, the background aleurone pigmentation is restricted to only the gown region. No differential Wx staining is observed between the endosperm underlying the pigmented and the nonpigmented aleurone areas (Figure 4.23C). This difference may likely have an explanation in the turnover rate of the post-transcriptional gene product. Early in endosperm development, a few starch granule bound enzymes may be produced and if the turnover rate of this enzyme is low, the enzyme will be available for a few cell generations for Wx starch production even if further transcription from the Wx does not occur. On the other hand, the gene product for the anthocyanin pathway may have a shorter life and availability is dependent on transcription in the cell where it is expressed.

5.2.7. Wx-wx twin sectors associated with excision of *En2*

The occurrence of Wx-wx twin sectors when the *wx-m 86246X* allele is present in one dose has been described in Section 4.2.6 (Figure 4.25). The presence of cellular structure (Figure 4.25 C and D) in the wx co-twin rules out this observation as an artifact during the staining procedure. The twin sectoring itself indicates the phenomenon is related to the excision process.

The presence of a Wx background in this genotype makes it possible to observe the wx sectors. In the absence of a Wx background expression, the wx sectors will remain invisible and would give the appearance of only isolated Wx sectors.

In the wx co-twin sector there is obviously a lack of Wx gene expression. Such a wx sector is similar to the expression with two doses of *En2*. One possibility is that following excision of *En2* from the *wx-m 86246X* allele during DNA replication there is a gain of one *En2* in one daughter cell and loss in the other. The cell which gained the transposed *En2* now has two copies; one at the *wx-m 86246X* allele and the other is the transposed *En2*. Two copies of *En2* in a cell can effectively suppress the background *Wx* gene expression. Progenies of these cells will appear adjacent to the Wx sector and will give the appearance of a twin sector. A gain of an extra element during the excision process has been

obvious with the *P-vv* studies (Greenblatt and Brink, 1962, 1963).

Another possibility is that the M function is not optimally operative in these cells carrying the *wx-m 86246X* allele. The source of the M function in these genotypes has been ascribed to either the activation of cryptic elements (Gierl et al., 1988a) or a hypothetical transcript of *En2* expressing the remnants of the ORF sequences as suggested in Section 5.2.3. In both cases, the product formed may not be the same as that formed by a standard *En*, i.e., the product may be similar to tnpD but not exactly tnpD. If such a defective product is responsible for the excisions observed in the genotypes carrying the *wx-m 86246X* allele, it may be expected that the excision process is not always precise. During excision, the defective transposase complex may not be able to protect the free termini formed at the site of excision (Saedler and Nevers, 1985). The unprotected termini are subjected to exonuclease degradation. The outcome of such a imprecise excision may result in one functional strand of DNA and a degraded nonfunctional strand. After replication, one daughter cell carrying the functional strand will be able to express the *Wx* gene and the other daughter cell carrying the defective strand will show wx expression giving rise to the observed twin sectors.

5.3. Somatic Transposition of *En*

In the maize kernel the starch producing endosperm tissue and the overlying aleurone tissue share a common lineage. Further, the aleurone tissue, differentiated to produce anthocyanin pigments, is an extension of the underlying endosperm (Randolph, 1936). The continuum of cell lineage between these tissues provides an ideal condition to study the destiny of *En* following excision in somatic tissue. The excision of *En* is monitored as wx-->Wx sectors in the endosperm using the *wx-844* allele. The fate of this excised *En* is monitored in the overlying aleurone using reporter alleles of anthocyanin marker genes.

Four reporter alleles have been used in this study. The *a-m(r)*, *a-m1* and the *a-m2 8004* alleles condition mutability at the *A* locus (Sections 3.1 and 3.5). The fourth, the *a2-m1* reporter allele conditions mutability at the *A2* locus (Sections 3.1 and 3.5). The mutable expression of the above alleles is observable in the aleurone layer.

The *a-m(r)* allele responds only to the M function of *En*. The presence of spots in a sector, therefore indicates an active M function. An absence of spots in an aleurone sector would indicate only a lack of the M function of *En*. The *a-m(r)* allele would therefore not be able to distinguish between complete lack of *En* and the presence of an *En* with

virtually no M activity, e.g., a weak *En* like one found in the *wx-m 86246X* allele with functional S product and questionable M action. Thus the argument suggests that a lack of spotting in a sector is not equivalent to a lack of *En* in that sector. Further, while studying the somatic events with the *wx-844* and the *a-m(r)* alleles (Section 4.3.1), it was observed that the *a-m(r)* allele itself is subject to change in somatic tissue. Therefore, some of the somatic events cannot be unambiguously decided as caused by events occurring at the *wx-844* allele.

The *a-m(r)* allele does not show distinct phenotypes with varied doses of *En*. Thus the use of the *a-m(r)* reporter allele would fail to detect in a somatic sector any variation in the number of *En*. In conclusion the *a-m(r)* allele is not a very suitable reporter allele to study the fate of excised *En* in somatic sectors.

The *a-m1* allele responds both to the S and the M functions of *En*. Absence of spots indicates absence of the M function and absence of supression of the background pale color (= presnce of pale coloration) indicates the absence of the S function of *En*. A lack of spots and a presence of pale background pigmentation in an aleurone sector can therefore be taken as absence of *En* in that sector. With these features, the *a-m1* reporter allele serves better than the *a-m(r)* allele in detecting lack of *En*. Yet, because this allele

responds to the M function of *En*, there is still a likelihood that it is subject to alteration even in a somatic sector. Variation in the dosage of *En* in a somatic sector is also difficult to detect with the *a-m1* allele. The disadvantages of both, the *a-m(r)* and the *a-m1* reporter alleles are eliminated by the use of the *a2-m1* reporter.

The *a2-m1* (state II) allele responds only to the S function of *En*. In the absence of *En* the *a2-m1* allele conditions a dark aleurone pigmentation and in the presence of *En* the pigmentation is suppressed. This is an ideal reporter allele to monitor the presence and absence of *En* in a somatic sector. Any aleurone pigmentation would indicate an absence of *En* activity in that region and a colorless aleurone would imply the presence of *En* in that region. Further, this allele is not subject to any alteration since it does not respond to the M function of *En* (McClintock, 1958; Menssen et al., 1990). The *a2-m1* reporter allele can also distinguish between one or more doses of *En* and this feature has made possible the detection of the twin sectors which has suggested a model for the frequent loss of *En* observed in somatic excision process.

5.3.1. The wx-->Wx sectors are due to excision of *En* from the *wx-844* allele and not due to inactivation of *En* at the *wx-844* allele

Precise to near precise excision of the *En element from the wx-844* allele would cause a Wx phenotype in the endosperm and will be observable as a wx-->Wx sector. Could inactivation of the *En* element at the *wx-844* allele cause the same phenotype? This proposition is based on the argument that inactivation will lead to abolition of tnpA production and a lack of binding of tnpA protein to the ends of *En* will allow transcription from the *Wx* gene to proceed. Further processing may remove the *En* sequences from the transcript resulting in a functional *Wx* mRNA.

The observations in this study contradict the inactivation hypothesis. When the *wx-844* allele is present in two or more doses the number of wx-->Wx sectors is significantly increased. If one copy of the *wx-844* allele is inactivated tnpA protein from the other copy should be able to prevent the hypothesized read-through transcription and therefore inactivation caused Wx sectors should rarely be observed. In fact, if the inactivation hypothesis were true, the number of Wx sectors observed when two doses of *wx-844* are present should be less than the number of Wx sectors observed when one dose of *wx-844* is present. Increased doses of the *wx-844* allele would cause an increased number of Wx sectors if the

Wx sectors were due to excision. This argument agrees with the observation.

5.3.2. Excision of *En* is always associated with the pale sectors of the *a-m1* allele and the colored sectors of the *a2-m1* allele

In Section 4.3.2 two types of aleurone spotting patterns are observed overlying a Wx sector when the *a-m1* reporter allele is used in combination with one dose of the *wx-844* allele. One is wx-->Wx in the endosperm and spotted with pale sectors in the aleurone (Table 11, #2). In this event excision of *En* from the *wx-844* allele has caused the wx-->Wx sector and the presence of *En* in this sector is indicated by the appearance of spots in the overlying aleurone. This event is therefore caused by reinsertion of the excised *En*. The other wx-->Wx sector observed is completely pigmented in the aleurone (Table 11, #3; Figure 4.29B). The pigmented aleurone indicates the absence of *En* activity in that sector after *En* has excised from the *wx-844* allele. No pale sector has been observed which is wx in the endosperm underneath. Therefore, all pale sectors observed with the *a-m1* allele (Figure 4.29) and the *wx-844* allele are related to excision of the *En*.

McClintock (1965a, 1971) has suggested that the pale sectors observed with the *a-m1* allele and the *Spm* element are

due to the inactivation of the *Spm* element in that sector. The observations in this study suggest that in these pale sector the *En* element is no longer present at the *wx* locus; it has excised from the *wx* locus. It is possible that after *En* has excised from the *wx-844* allele it has reinserted at another location and is inactivated at the new site due to methylation. This argument also explains the loss of *En* activity in the pale Wx sectors (Table 11, #3; Figure 4.29). An alternative possibility is that the *En* has not reinserted after excision.

A similar situation is encountered with the colored aleurone sectors (Figure 4.31) observed with the *a2-m1* and the *wx-844* alleles. In Section 4.3.4 an association between the colored aleurone sectors and the Wx endosperm sectors has been established. McClintock (1965a, 1965b, 1971) has also suggested inactivation of the *Spm* element in these colored sectors. Our study does not support the model of inactivation of *En* at the *wx-844* allele as a cause of the sectors that lack *En* activity. There is a possibility that *En* is inactivated at the new location after transposition but, for certain, the *En* element is not inactivated at the *wx-844* allele in these sectors. This is because, the wx-->Wx sectors are associated with aleurone sectors showing a lack of *En* activity and these wx-->Wx sectors are caused by the

excision of *En* from the *wx-844* allele and not due to inactivation of *En* at the *wx-844* allele.

The *a-m1* pale sectors and the *a2-m1* colored sectors are frequently observed and therefore, the cause of such excision related lack of *En* activity is a very common phenomenon. Lack of reinsertion or inactivation at the new location can justify such observations. But, why *En* should frequently be inactivated at a new location or fail to reinsert after excision is difficult to comprehend. Again, the observations on 'loss and gain' type of twin sectors (Figure 4.32) are not explained by the lack of reinsertion or post transpositional inactivation models.

If transposition of *En* is assumed to occur during chromosome replication, the *En* loss events can easily be explained. Figure 5.3 describes the possible transposition events that result in loss of *En*. If transposition occurs from a replicated region to the sister chromatid (Figure 5.3 ,#1), one strand will be recovered carrying the Wx revertant allele and lacking the *En* element (Figure 5.3, 1a). Transposition of *En* from a replicated region of one chromosome to a replicated region in another chromosome (Figure 5.3, #2) would also lead to the recovery of a daughter cell lacking the *En* element after mitotic segregation of chromatids is completed and if the chromatid carrying the revertant Wx allele in one chromsome cosegregates with the chromatid lacking the *En*

element in the other chromosome (Figure 5.3, 2a). Transposition of *En* from an unreplicated region to a replicated region in the same (Figure 5.3, #3) chromosome or to a replicated region of a different chromsome (Figure 5.3, #4) also leads to the recovery of a daughter cell that lack the *En* element (Figure 5.3, 3b and 4a).

Based on the observations on twin sectors (Section 4.3.5) a model is proposed where *En* transposition occurs, after the chromosome segment carrying the *En* element has completed replication and the model eliminates the need to assume that transposition occurs from an unreplicated segment of a chromosome (Figure 5.3, #3 and #4). The model and its rationale is explained in the next section. This model explains all the observed types of sectors found in this study.

5.3.3. Association *En* transposition with chromosome replication

The observations of somatic events with the *wx-844* and the *a2-m1* allele provide clues to the timing of transposition during cell cycle. This is because of the unique advantage of the *a2-m1* allele to respond to the dosage of *En*. The *a2-m1* allele shows a coarse aleurone sectoring pattern in the presence of one dose of *En*. In the presence of two doses of

En, the aleurone pattern is reduced to a finer pattern, i.e., smaller and fewer pigmented regions in the aleurone. Increased dosage of *En* causes further fineness in the aleurone pigmentation pattern and to the extent that the aleurone becomes colorless in the presence of four or more doses of *En*.

To decide the destiny of *En* after excision, Wx sectors have been examined for the overlying aleurone pigmentation pattern. A colored aleurone above the Wx sector suggests the absence of *En* in that sector. A coarse pigmentation pattern suggests that only one *En* is present in that sector and fine pigmentation pattern is indicative of the presence of more than one *En*. The location of *En* in the sector examined is decided on the basis of the presence of wx mutability in the endosperm. A wx mutable sector indicates that *En* is still present at the *wx-844* allele and a Wx endosperm sector indicates that *En* has excised from the *wx-844* allele.

The nature of the sectors observed have been described in Sections 4.3.4 and 4.3.5. In Section 4.3.5, the occurrence of twin sectors has been described. The 'loss and gain' type of twin sectors has been most frequently observed.

In this type of twins, colored sectors were found juxtaposed to fine sectors (Figure 4.32). In these sectors the colored sectors were Wx and the fine sectors were wx mutable

(Figure 4.33). Because of their twin nature one can conclude that the same event led to the gain of one *En* in one daughter cell giving rise to the fine sector and the same event also caused loss of the *En* the other daughter cell giving rise to the colored sector. The colored co-twin sector is Wx and therefore, the twin event is a consequence of an excision event of the *En* element from the *wx-844* allele. The event includes excision of *En* in one daughter cell while retaining an intact *wx-844* allele in the other daughter cell. This is possible if at the time of excision the chromosomal region that includes the *En*-containing *Wx* locus has already replicated and excision occurred in one of the sister chromatid (Figure 5.4). This process would terminate in one daughter cell carrying the revertant *Wx* allele (Figure 5.4, 2a) and the other daughter cell an intact *wx-844* allele (Figure 5.4, 2b). The additional information the twin sector provides is that the daughter cell carrying the revertant *Wx* allele lacks *En* activity (Figure 5.4, 2a) where as the other daughter cell carrying the intact *wx-844* allele reveals the gain of an *En* element (Figure 5.4, 2b). This loss and gain of *En* can be explained if it is assumed that the excised *En* became reinserted in an already replicated segment of the sister chromatid carrying the *wx-844* allele and not in the same chromatid from which excision has occurred (Figure 5.4, #2). Thus, the occurrence of 'loss and gain' type of twin sectors can be ex-

plained if transposition of *En* from the *wx-844* allele is assumed to occur during chromosome replication.

A very few isolated fine sectors showed, adjacent to them, a Wx area with coarse spotting in the aleurone and is shown in Figure 4.34B. Assuming that these adjacent occurring sectors are twin events one can describe them as 'gain and no loss' sectors as opposed to the 'loss and gain' sectors described previously.

In the 'gain and no loss' types, one of the co-twins is Wx endosperm with coarse aleurone pattern and the other co-twin is wx-mutable endosperm with fine aleurone pattern. This can arise from a single transposition event during chromosome replication if excision of *En* occurs in one of the chromatids after replication of that chromosome segment and reinsertion occurs in an unreplicated region of the chromosome (Figure 5.4, #3). After replication is completed, the chromatid from which excision occurred would carry only the transposed *En* (Figure 5.4, 3a) and the other sister chromatid would carry both the transposed *En* and the one already existing at the *wx-844* allele (Figure 5.4, 3b).

This model, suggesting that transposition occur from a replicated chromosome fragment, explains all the observed types of sectors. The explanation for the isolated and individual sectors observed, follows.

On the basis of the observations on the individual sectors it was concluded that four types of outcomes are present. One is excision of *En* from the *wx-844* allele and presence of one *En* in the sector. These are the Wx endosperm sectors with a coarse aleurone pattern (Figure 4.35, #3; Table 12, #2). The event that has resulted in this sector can be described as excision of *En* followed by its reinsertion somewhere in the genome, a normal transposition event. On the basis of the model presented in Figure 5.4, reinsertion of *En* in the same chromatid (Figure 5.4, #1a) or reinsertion in an unreplicated region of a chromosome (Figure 5.4, #3a) would result in a normal transposition of event, i.e., excision and recovery of one *En*- 'no-loss no-gain' event.

The second type is excision of *En* from the *wx-844* allele and no *En* activity in that sector. These are the Wx endosperm sectors with a completely colored aleurone (Figure 4.35, #5; Table 12, #5). This is described as an *En* loss event. If transposition occurs during chromosome replication, the *En* loss event would result from transposition to the sister chromatid (Figure 5.4, #2a) and not to the same chromatid from which excision occurred. The third type is the gain of an *En* in a sector while the *En* at the *wx-844* allele is still present. These are the sectors with a wx mutable endosperm and a fine aleurone pigmentation (Figure

4.35, #2; Table 12, #3). This outcome would result only if transposition occurs during chromosome replication. Excision of *En* from one of the replicated chromatids followed by its insertion in the sister chromatid (Figure 5.4, 2b) or in an unreplicated region (Figure 5.4, 3b) would lead to the recovery in the sister chromatid two *En*s, one at the *wx-844* allele and the other being the transposed *En*. The fourth type of sector observed is the one with a Wx endosperm and a fine aleurone pigmentation (Figure 4.35, #4; Table 12, #4). This pattern would result from a secondary transposition event. One round of normal transposition (Figure 5.4, 1a or 3a) followed by an event just described for the fine type of aleurone (Figure 5.4, 2b or 3b) would lead to a Wx endosperm and a fine aleurone. Thus all observed sectors are explained by the model that invokes that transposition takes place from a replicated region of the chromosome during DNA replication.

The transposition of the *Ac* element also occurs during chromosome replication and transposition from an already replicated region to a region that has not yet replicated and also to an already replicated region is extensively documented (Greenblatt, 1966, 1968, 1974, 1984; Greenblatt and Brink, 1963) and has been discussed in Section 2.7.

On the basis of frequent observations of loss events it is concluded that *En* transposes during DNA replication and the most frequent mode is transposition from an already

replicated strand to the another location in the opposite strand which has also undergone replication. But it is not proposed that it is the sole mode of transposition. Transposition to the same chromatid from which excision occurred, and to unreplicated region also occurs.

Chromosome replication and transposition has also been associated with the Tn10 transposon (Roberts et al., 1985). In this case it has been shown that the Tn10 transposase prefers hemimethylated DNA for excision. Avraham and Walbot (1990) have also reported the increase of excision events during proliferation of the aleurone. These observations support the conclusion from this study that *En* is most vulnerable to transposition during DNA replication. Most likely the *En* encoded transposase acts preferentially on hemimethylated regions of DNA which is at a maximum level immediately after the passage of of the replication fork and before the action of methylating enzymes.

Figure 5.1 The structure of the *I-102* element

The 3697 bp long *I-102* is derived from the *En1* sequence by an internal deletion of 4590 bp removing nucleotides 1862-6451 of *En1*. The *I-102* element retains the intact termini structure. The 1.8 kb transcript specifies the tnpR product. Exon1 and exons 7-11 of tnpA (open boxes in *En1* structure) are shared by the tnpR (open boxes in *I-102* structure). The exon2 of tnpR is derived from the ORF1 and is not represented in the *En1* encoded tnpA. Exons 2-6 of tnpA are represented in tnpR. Thus tnpR shares a common carboxy-terminal domain (the last 5 exons) with tnpA but differs in its amino terminal (the first two exons).

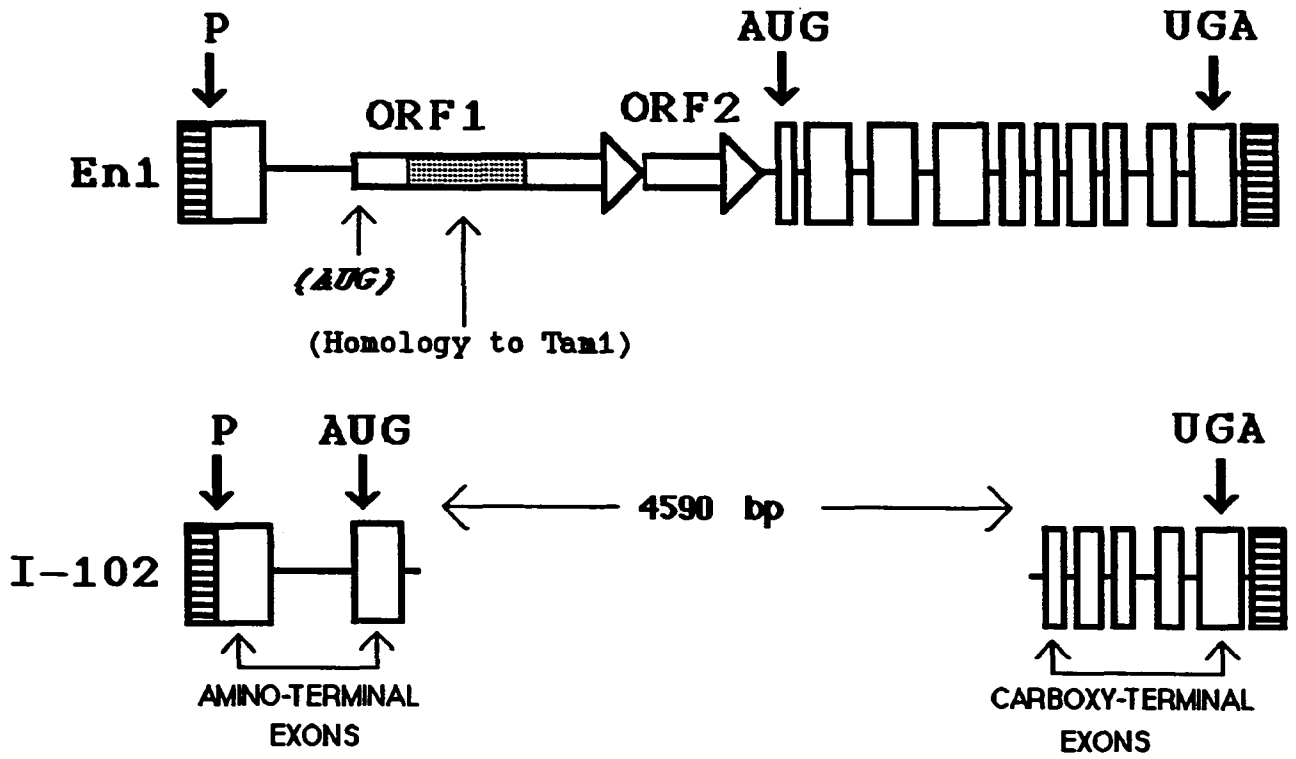

P
AUG
UGA
ORF1
ORF2
En1
(AUG)
(Homology to Tam1)
P
AUG
UGA
4590 bp
I-102
AMINO-TERMINAL
EXONS
CARBOXY-TERMINAL
EXONS

Figure 5.2 The gene structure of *En2*

En2 **cloned from the *wx-m 86246X* allele bears a deletion of 1126 bp deletion from *En1* as shown. The deletion includes part of the ORFs but it retains all the exon (1 to 11 and shown here as open boxes) sequences necessary for the 2.5 kb transcript. *En2* produces tnpA at a five fold reduced level as compared to lines carrying the *wx-844* allele. The part of ORF1 that shows homology to *Tam1* element is also retained in the structure of *En2*. The termini of this element also remain intact.**

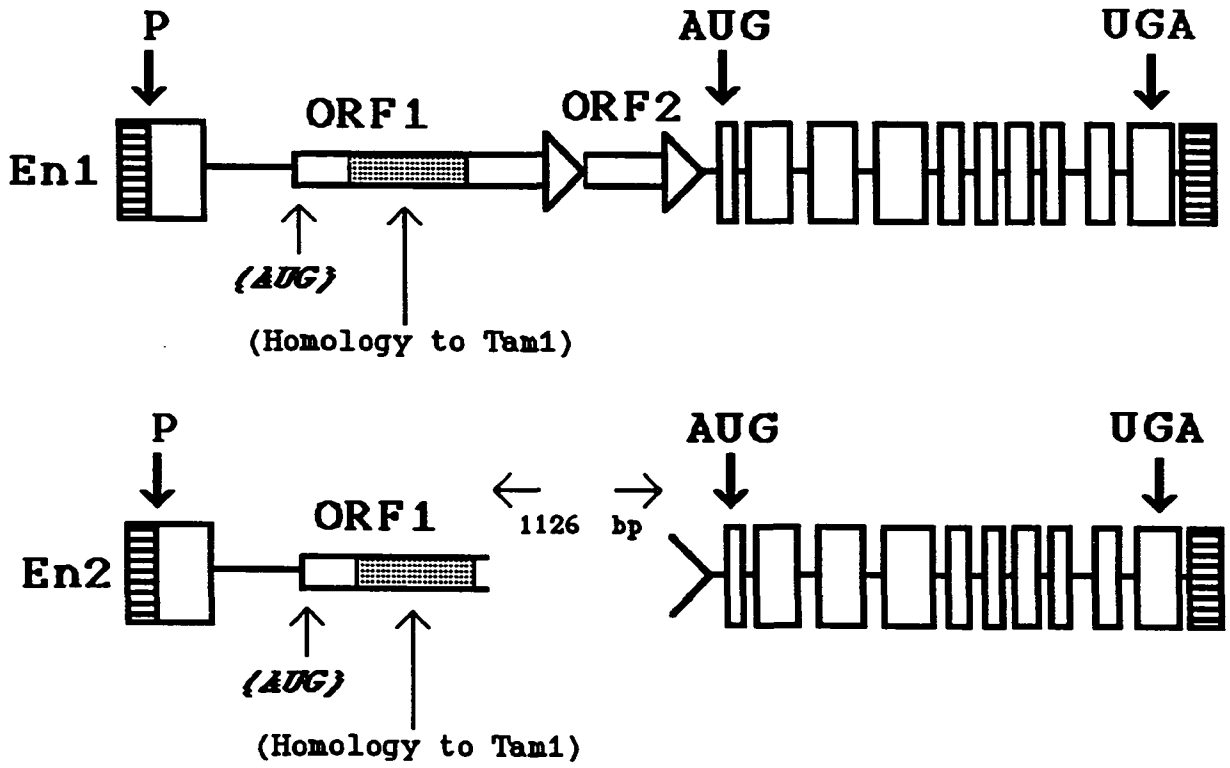

P
AUG
UGA
ORF1
ORF2
En1
(AUG)
(Homology to Tam1)
P
AUG
UGA
ORF1
1126 bp
En2
(AUG)
(Homology to Tam1)

Figure 5.3 Transposition of *En* during chromosome replication explains *En* loss events following excision

1. Transposition from a replicated chromatid to the sister chromatid. No *En* is recoverd in the strand (1a) carrying the revertant Wx allele

2. Transposition from a replicated chromatid to the replicated region of another chromosome. After random mitotic segregation of chromatids, the chromatid carrying the revertant Wx allele and the chromatid lacking *En* in the other chromosome may co-segregate to a daughter cell (2a). This cell if develops into a sector would be observed as an *En* loss event.

3. Transposition of *En* from an unreplicated segment to a replicated region in the same chromosome. One strand (3b) would always be recovered lacking the *En* element.

4. Transposition of *En* from an unreplicated segment to a replicated region in another chromosome. After random mitotic segregation of chromatids the strand carrying the revertant Wx allele may co-segregate with the other chromosome lacking *En* (4a) to form a cell lineage that would be observed as an *En* loss event.

Loss of *En* Following Excision

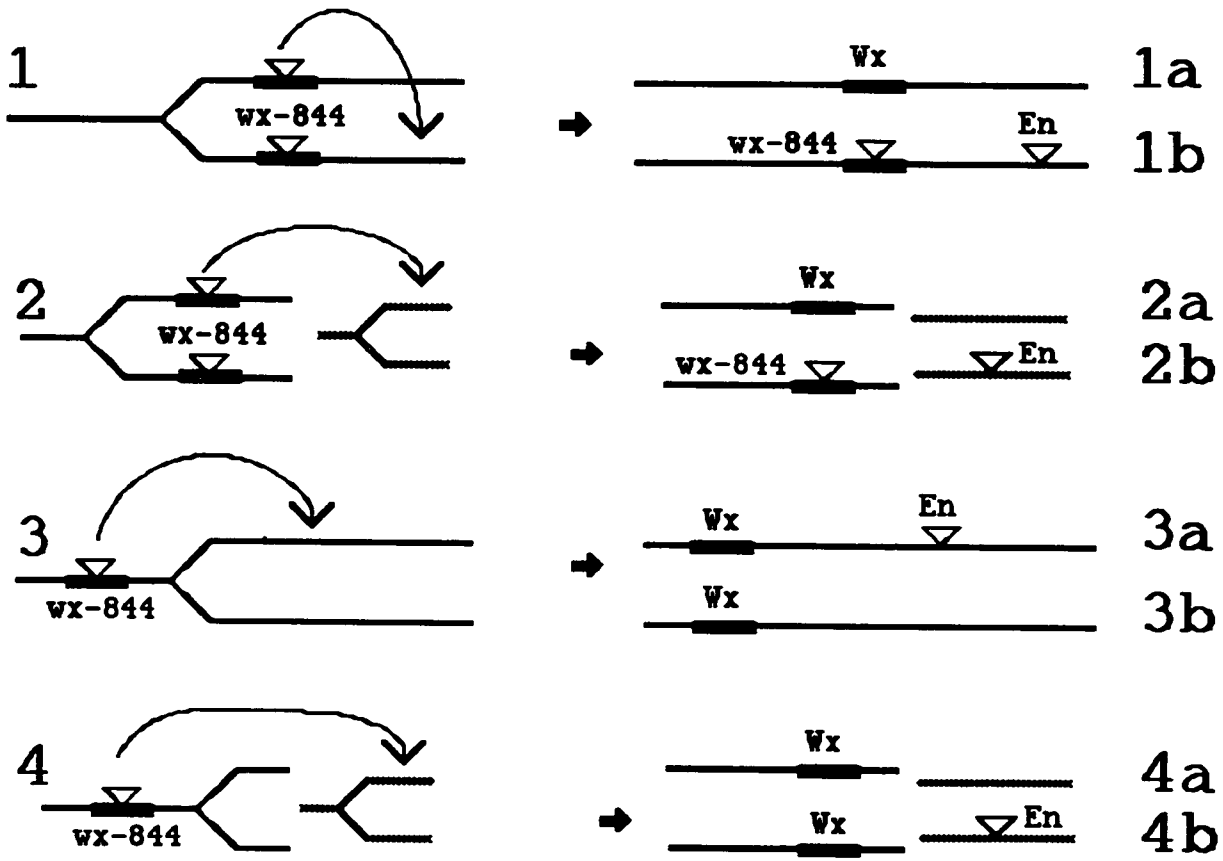


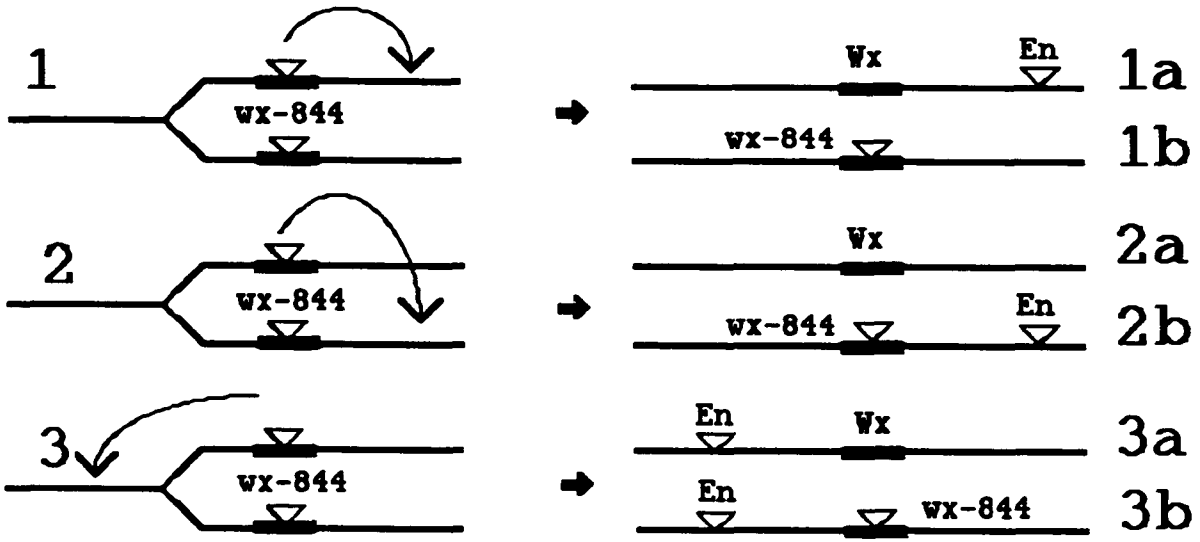
Figure 5.4 Model of *En* transposition during chromosome replication

1. Transposition from a replicated chromatid to the same chromatid. One strand (1a) recovers the revertant Wx allele with the transposed *En*. The other strand (1b) is identical to the original.

2. Transposition from a replicated chromatid to the sister chromatid. No *En* is recoverd in the strand (2a) carrying the Wx allele whereas an extra *En* is recovered in the other strand (2b) in addition to *En* at the *wx-844* allele. This is the most frequent mode observed in this study.

3. Transposition from a replicated chromatid to an unreplicated region. In this case both the strands contain the transposed *En*. This type of event has been observed less frequently in this study.

TRANSPOSITION OF *En*
during chromosome replication



6. LITERATURE CITED

Avraham, A. L., and V. Walbot. 1990. Regulation of the timing of transposable element excision during maize development. Science 248:1534-1537.

Banks, J., J. Kingsbury, V. Raboy, J. W. Schiefelbein, O. Nelson, and N. V. Fedoroff. 1985. The *Ac* and *Spm* controlling element families in maize. Cold Spring Harbor Symp. Quant. Biol. 50:307-311.

Breathnach, R., and P. Chambon. 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu. Rev. Biochem. 50:349-383.

Brink, R. A., and R. A. Nilan. 1952. The relation between light variegated and medium variegated pericarp in maize. Genetics 37:519-544.

Chen, J., I. M. Greenblatt, and S. L. Dellaporta. 1987. Transposition of *Ac* from the *P* locus of maize into unreplicated chromosomal sites. Genetics 117:109-116.

Coe, E. H., and G. M. Neufer. 1977. The genetics of corn. Pp. 111-223. In Sprague, G. F. (ed.) Corn and Corn Improvement. The American Society of Agronomy Inc., Madison, WI.

Cuypers, H., S. Dash, P. A. Peterson, H. Saedler, and A. Gierl. 1988. The defective *En-I102* element encodes a product reducing the mutability of the *En/Spm* transposable element system of *Zea mays*. EMBO J. 7:2953-2960.

Dooner, H. K. 1983. Coordinated genetic regulation of flavanoid biosynthetic enzymes in maize. Mol. Gen. Genet. 189:136-141.

Döring, H. -P., and P. Starlinger. 1986. Molecular genetics of transposable elements in plants. Annu. Rev. Genet. 20:175-200.

Echt, C. S. and D. Schwartz. 1981. Evidence for the inclusion of controlling elements within the structural gene at the *waxy* locus in maize. Genetics 99:275-284.

Emerson, R. A. 1917. Genetical studies of variegated pericarp of maize. Genetics 2:1-35.

Fedoroff, N. 1983. Controlling elements in maize. Pp. 1-63. In Shapiro, J. (ed.) Mobile Genetic Elements. Academic Press, New York.

Fedoroff, N. 1989. Maize transposable elements and development. Cell 56:181-191.

Fedoroff, N., and J. Banks. 1988. Is the Suppressor-mutator element controlled by a basic developmental regulatory mechanism. Genetics 120:559-578.

Fincham, J. R. S., and G. R. K. Sastry. 1974. Controlling elements in maize. Annu. Rev. Genet. 8:15-50

Freeling, M. 1984. Plant transposable elements and insertion sequences. Annu. Rev. Plant Physiol. 35:277-298.

Frey, M., S. M. Tavantzis, and H. Saedler. 1989. The maize *En-I/Spm* element transposes in potato. Mol. Gen. Genet. 217:172-177.

Friedemann, P., and P. A. Peterson. 1982. The *Uq* controlling element system in maize. Mol. Gen. Genet. 187:19-29.

Gierl, A., Zs. Schwarz-Sommer, and H. Saedler. 1985. Molecular interactions between the components of the *En-I* transposable element system of *Zea mays*. EMBO J. 4:579-583.

Gierl, A., H. Cuypers, S. Lutticke, A. Pereira, Zs. Schwarz-Sommer, S. Dash, P. A. Peterson, and H. Saedler. 1988a. Structure and function of the *En/Spm* transposable element system of *Zea mays*: Identification of the suppressor component of *En*. Pp. 115-119. In Nelson, O. (ed.) Proc. Intl. Symp. on Plant Transposable Elements. Plenum, New York.

Gierl, A., S. Lutticke, and H. Saedler. 1988b. *TnpA* product encoded by the transposable element *En-1* of *Zea mays* is a DNA binding protein. EMBO J. 7:4045-4053.

Gierl, A., H. Saedler, and P. A. Peterson. 1989. Maize transposable elements. Annu. Rev. Genet. 23:71-85.

Gierl, A., and H. Saedler. 1989. Transposition in plants. Pp. In Eckstein, F., and D. M. J. Lilley (ed.) Nucleic Acids and Molecular Biology. Springer, Heidelberg.

Gonella, J. A., and P. A. Peterson. 1977. Controlling elements in a tribal maize from Colombia: *Fcu*, a two unit system. Genetics 85:629-645.

Gonella, J. A., and P. A. Peterson. 1978. The *Fcu* controlling element system in maize: II. On the possible heterogeneity of controlling elements. III. On the variable dilute pigmenting capacity of *r-cu*. Mol. Gen. Genet. 167:29-36.

Grant, S. R., A. Gierl, and H. Saedler. 1990. *En/Spm* encoded *tnpA* protein requires a specific target sequence for suppression. EMBO J. 9:2029-2035.

Greenblatt, I. M. 1966. Transposition and replication of Modulator in maize. Genetics 53:361-369.

Greenblatt, I. M. 1968. The mechanism of Modulator transposition in maize. Genetics 58:585-597.

Greenblatt, I. M. 1974. Movement of Modulator in maize: a test of an hypothesis. Genetics 77:671-678.

Greenblatt, I. M. 1984. A chromosome replication pattern deduced from pericarp phenotypes resulting from movements of transposable element, Modulator, in maize. Genetics 108:471-485.

Greenblatt, I. M., and R. A. Brink. 1962. Twin mutations in medium variegated pericarp in maize. Genetics 47:489-501.

Greenblatt, I. M., and R. A. Brink. 1963. Transposition of Modulator in maize into divided and undivided chromosome segments. Nature 197:412-413.

Hehl, R., H. Sommer, and H. Saedler. 1987. Interaction between the *Tam1* and *Tam2* transposable elements of *Antirrhinum majus*. Mol. Gen. Genet. 207:47-53.

Kermicle, J. L. 1978. Imprinting of gene action in maize endosperm. Pp. 357-371. In Walden, D. B. (ed.) Maize Breeding and Genetics. John Wiley and Sons, Inc., New York.

Kim, H. -Y., J. W. Schiefelbein, V. Raboy, D. B. Furtek, and O. E. Nelson. 1987. RNA splicing permits expression of a maize gene with a defective Suppressor-mutator transposable element insertion in an exon. Proc. Natl. Acad. Sci., USA 84:5863-5867.

Klösgen, R. B., A. Gierl, Zs. Schwarz-Sommer, and H. Saedler. 1986. Molecular analysis of the *waxy* locus of *Zea mays*. Mol. Gen. Genet. 203:237-244.

Laski, F. A., D. C. Rio, and G. M. Rubin. 1986. Tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. Cell 44:7-19.

Masson, P., R. Surosky, J. Kingsbury, and N. Fedoroff. 1987. Genetic and molecular analysis of the *Spm*-dependent *a-m2* alleles of the maize *a* locus. Genetics 177:117-137.

McClintock, B. 1946. Maize Genetics. Carnegie Inst. of Wash. Year Book 45:176-186.

McClintock, B. 1947. Cytogenetic studies in maize and Neurospora. Carnegie Inst. of Wash. Year Book 46:146-152.

McClintock, B. 1948. Mutable loci in maize. Carnegie Inst. of Wash. Year Book 47:155-169.

McClintock, B. 1950a. Mutable loci in maize. Carnegie Inst. of Wash. Year Book 49:157-167.

McClintock, B. 1950b. The origin and behaviour of mutable loci in maize. Proc. Natl. Acad. Sci. 36:344-355.

McClintock, B. 1951. Chromosome organization and genic expression. Cold Spring Harbor Symp. Quant. Biol. 16:13-47.

McClintock, B. 1952. Mutable loci in maize. Carnegie Inst. of Wash. Year Book 51:212-219.

McClintock, B. 1954. Mutations in maize and chromosomal abberations in Neurospora. Carnegie Inst. of Wash. Year Book 53:254-260.

McClintock, B. 1955. Controlled mutation in maize. Carnegie Inst. of Wash. Year Book 54:245-255.

McClintock, B. 1956. Mutation in maize. Carnegie Inst. of Wash. Year Book 55:323-332.

McClintock, B. 1957. Genetic and cytogenetic studies of maize. Carnegie Inst. of Wash. Year Book 56:393-401.

McClintock, B. 1958. The suppressor-mutator system of control of gene action in maize. Carnegie Inst. of Wash. Year Book 57:415-429.

McClintock, B. 1961a. Further studies of the suppressor mutator system of control of gene action in maize. Carnegie Inst. of Wash. Year Book 60:469-476.

McClintock, B. 1961b. Some parallels between gene control systems in maize and bacteria. Am. Nat. 95:265-277.

McClintock, B. 1962. Topographical relations between elements of control systems in maize. Carnegie Inst. of Wash. Year Book 61:448-461.

McClintock, B. 1963. Further studies of gene control systems in maize. Carnegie Inst. of Wash. Year Book 62:486-493.

McClintock, B. 1964. Aspects of gene regulation in maize. Carnegie Inst. of Wash. Year Book 63:592-602.

McClintock, B. 1965a. The components of action of the regulators *Spm* and *Ac*. Carnegie Inst. of Wash. Year Book 64:527-536.

McClintock, B. 1965b. The control of gene action in maize. Brookhaven Symp. Biol. 18:162-184.

McClintock, B. 1967. The states of a gene locus in maize. Carnegie Inst. of Wash. Year Book 66:664-671.

McClintock, B. 1968. The states of a gene locus in maize. Carnegie Inst. of Wash. Year Book 66:20-28.

McClintock, B. 1971. The contribution of one component of a contro system to versatility of gene expression. Carnegie Inst. of Wash. Year Book 70:5-17.

Menssen, A., S. Hohmann, W. Martin, P. S. Schnable, P. A. Peterson, H. Saedler, and A. Gierl. 1990. The *En/Spm* transposable element of *Zea mays* contains splice sites at the termini generating a novel intron from a *dSpm* element in the *A2* gene. EMBO J. 9:3051-3057.

Nelson, O. E., and A. S. Klein. 1984. Characterization of an *Spm* controlled bronze-mutable allele in maize. Genetics 106:769-779.

Nevers, P., and H. Saedler. 1977. Transposable genetic elements as agents of gene instability and chromosome rearrangements. Nature 268:109-115.

Nevers, P., N. A. Shepherd, and H. Saedler. 1986. Plant transposable elements. Adv. Bot. Res. 12:102-203.

Nowick, E. M., and P. A. Peterson. 1981. Transposition of the Enhancer controlling element system of maize. Mol. Gen. Genet. 183:440-448.

O'Reilly, C., N. S. Shepherd, A. Pereira, Zs. Schwarz-Sommer, I. Bertram, D. S. Robertson, P. A. Peterson, and H. Saedler. 1985. Molecular cloning of the *a1* locus of *Zea mays* using the transposable elements *En* and *Mu1*. EMBO J. 4:877-882.

Paz-Ares, J., U. Wienand, P. A. Peterson, and H. Saedler. 1986. Molecular cloning of the *c* locus of *Zea mays*: a locus regulating the anthocyanin pathway. EMBO J. 5:829-833.

Pereira, A., Zs. Schwarz-Sommer, A. Gierl, I. Bertram, P. A. Peterson, and H. Saedler. 1985. Genetic and molecular analysis of the Enhancer (*En*) transposable element system of *Zea mays*. EMBO J. 4:17-23.

Pereira, A., H. Cuypers, A. Gierl, Zs. Schwarz-Sommer, and H. Saedler. 1986. Molecular analysis of the *En-Spm* transposable element system of *Zea mays*. EMBO J. 5:835-841.

Pereira, A., and H. Saedler. 1989. Transpositional behaviour of the maize *En/Spm* element in transgenic tobacco. EMBO J. 8:1315-1321.

Peterson, P. A. 1953. A mutable pale green locus in maize. Genetics 38:682-683.

Peterson, P. A. 1957. A new mutable at the *A1* locus in maize (*a1m*). Genetics 42:388-389.

Peterson. P. A. 1960. The pale green mutable system in maize. Genetics 45:115-133.

Peterson, P. A. 1961. Mutable *a1* of the *En* system in maize. Genetics 46:759-771.

Peterson, P. A. 1965. A relation between the *Spm* and *En* control systems in maize. Am. Nat. 99:391-398.

Peterson, P. A. 1966. Phase variation of regulatory elements in maize. Genetics 54:249-266.

Peterson, P. A. 1970. The *En* mutable system of maize. III. Transposition associated with mutational events. Theor. Appl. Genet. 40:367-377.

Peterson, P. A. 1976a. Change in state following transposition of a regulatory element of the enhancer system in maize. Genetics 84:469-483.

Peterson, P. A. 1976b. Basis for the diversity of states of controlling elements of maize. Mol. Gen. Genet. 149:5-21.

Peterson, P. A. 1978. Controlling elements: The induction of mutability at the *A2* and *C* loci in maize. Pp. 601-631. In Walden, D. B. (ed.) Maize Breeding and Genetics. John Wiley and Sons, Inc., New York.

Peterson, P. A. 1985. The isolation of *En-1* in the *wx-844* allele. Maize Genet. Coop. Newsl. 59:3.

Peterson, P. A. 1986. Mobile elements in maize: A force in evolutionary and plant breeding process. Stadler Genet. Symp. 17:47-78.

Peterson, P. A. 1987. Mobile elements in plants. CRC Crit. Rev. Plant Sci. 6:105-208.

Randolph, L. F. 1936. Developmental morphology of the caryopsis in maize. J. Agric. Res. 53:881-916.

Reddy, A. R., and P. A. Peterson. 1983. Transposable elements of maize: Genetic basis of pattern differentiation of some mutable *c* alleles of the Enhancer system. Mol. Gen. Genet. 192:21-31.

Reddy, L. V., and P. A. Peterson. 1984. Enhancer transposable element induced changes at the *A* locus in maize: the *a-m1 6078* allele. Mol. Gen. Genet. 194:124-137.

Reddy, L. V., and P. A. Peterson. 1985. *Spm* and *I* element changes with the *a-m2 8004* allele in maize. Mol. Gen. Genet. 200:211-219.

Rhoades, M. M. 1938. Effect of the *Dt* gene on the mutability of the *a1* allele of maize. Genetics 23:377-397.

Rhodes, P. R., and L. O. Vodkin. 1985. Highly structured sequence homology between an insertion element and the gene in which it resides. Proc. Natl. Acad. Sci. USA 82:493-497.

Rhodes, P. R., and L. O. Vodkin. 1988. Organization of the *Tgm* family of elements in soybean. Genetics 120:597-604.

Rio, D. C., F. A. Laski, and G. M. Rubin. 1986. Identification and immunochemical analysis of biologically active *Drosophila* P element transposase. Cell 44:21-32.

Roberts, D., B. C. Hoopes, W. R. McClure, and N. Kleckner. 1985. *IS10* transposition is regulated by DNA adenine methylation. Cell 43:117-130.

Robertson, D. S. 1978. Characterization of a mutator system in maize. Mutat. Res. 51:21-28.

Saedler, H., and P. Nevers. 1985. Transposition in plants: a molecular model. EMBO J. 4:585-590.

Schiefelbein, J. W., V. Raboy, N. V. Fedoroff, and O. Nelson. 1985. Deletions with a defective Suppressor-mutator element in maize affect the frequency and developmental timing of its excision from the bronze locus. Proc. Natl. Acad. Sci. (USA) 82:4783-4787.

Schiefelbein, J. W., V. Raboy, H. Kim, and O. Nelson. 1988. Molecular characterization of Suppressor-mutator (*Spm*)-induced mutations at the *bronze-1* locus in maize: the *bz-m 13* alleles. Pp. 261-278. In Nelson, O. (ed.) Proc. Intl. Symp. Plant Transposable Elements. Plenum, New York.

Schnable, P. S., and P. A. Peterson. 1986. Distribution of genetically active *Cy* elements among diverse maize lines. Maydica 31:59-81.

Schwarz-Sommer, Zs., A. Gierl, R. B. Klösgen, U. Wienand, P. A. Peterson, and H. Saedler. 1984. The *Spm* (*En*) transposable element controls the excision of a 2 kb DNA insert at the *wx-m8* locus of *Zea mays*. EMBO J. 3:1021-1028.

Schwarz-Sommer Zs., A. Gierl, H. Cuypers, P. A. Peterson, and H. Saedler. 1985a. Plant transposable elements generate the sequence diversity needed in evolution. EMBO J. 4:591-597.

Schwarz-Sommer, Zs., A. Gierl, R. Berntgen, and H. Saedler. 1985b. Sequence comparison of "states" of *a1-m1* suggests a model of *Spm* (*En*) action. EMBO J. 4:2439-2443.

Schwarz-Sommer, Zs., N. Shepherd, E. Tacke, A. Gierl, W. Rohde, L. Leclercq, M. Mattes, R. Berndtgen, P. A. Peterson, and H. Saedler. 1987. Influence of transposable elements on the structure and function of the *A1* gene of *Zea mays*. EMBO J. 6:287-294.

Schwarz-Sommer, Zs., and H. Saedler. 1988. Transposons and retrotransposons in plants: Analysis and biological relevance. Pp. 343-354. In Kingsman, A. J., S. M. Kingsman, and K. F. Chatter (ed.) Transposition. Cambridge Univ. Press, Cambridge.

Shepherd, N. S., Zs. Schwarz-Sommer, U. Wienand, H. Sommer, B. Deumling, P. A. Peterson, and H. Saedler. 1982. Cloning of a genomic fragment carrying the insertion element *Cin1* of *Zea mays*. Mol. Gen. Genet. 188:266-271.

Shure, M., S. Wessler, and N. Fedoroff. 1983. Molecular identification and isolation of the waxy locus in maize. Cell 35:225-233.

Sommer, H., R. Hehl, E. Krebbers, R. Piotrowiak, W. E. Lönning, and H. Saedler. 1988. Transposable elements of *Antirrhinum majus*. Pp 227-235. In Nelson, O. (ed.) Plant Transposable elements. Proc. Intl. Symp. Plant Transposable Elements. Plenum, New York.

Tacke, E., Zs. Schwarz-Sommer, P. A. Peterson, and H. Saedler. 1986. Molecular analysis of "states" of the *A1* locus of *Zea mays*. Maydica 31:83-91.

Van Schaik, N. W., and R. A. Brink. 1959. Transposition of Modulator, a component of the variegated pericarp allele in maize. Genetics 44:725-738.

Wienand, U., U. Weydemann, U. Niesbach-Klösgen, P. A. Peterson, and H. Saedler. 1986. Molecular cloning of the *C2* locus of *Zea mays*- the gene coding for chalcone synthase. Mol. Gen. Genet. 203:202-207.

APPENDIX

Source of Kernels Used for Analysis of Somatic Sectors

A. Source of kernels for Sections 4.3.1 (*wx-844* with *a-m(r)*)

86 0131/0201-4, 86 0132/0201-6, 86 0132/0201-7,
86 0132/0203T
[Cross: *a/a wx/wx* X *a-m(r)/a-m1 wx-844/wx*]

(*a/a wx/wx*) X 87 5340, 5343Y, 5345Y, 5347W, 5347Y, 5349X
[Cross: *a/a wx/wx* X *a-m(r)/a wx-844/wx*: The *a/a wx/wx* tester used here was from various ranges in the field]

(*a/a wx/wx*) X 87 1359, 1360
(*a/a wx/wx*) X 88 0602, 0603
[Cross: *a/a wx/wx* X *a-m(r)/a-m(r) wx-844/wx-844*: The *a/a wx/wx* tester used here was from various ranges in the field]

B. Source of kernels for Section 4.3.2 (*wx-844* with *a-m1*)

86 0131/0201-4, 86 0132/0201-6, 86 0132/0201-7,
86 0132/0203T
[Cross: *a/a wx/wx* X *a-m(r)/a-m1 wx-844/wx*]

C. Source of kernels for Section 4.3.3 (*wx-844* with *a-m2 8004*)

87 1142-1150/1223-1230

[Cross: *a-m2 8004/a-m2 8004 wx/wx* X *a/a wx-844/wx*]

D. Source of kernels for Sections 4.3.4 and 4.3.5 (*wx-844* with *a2-m1*)

86 0351-0360/0601-0606 (Used for analysis of individual sectors)

87 1212-1222, 0941-0942 / 1233X, 1235X, 1236X, 1237Y, 1237Z, 1238X, 1240, 1242Z, 1243Y (Used for analysis of individual as well as twin sectors)

[Cross: *a2-m1/a2-m1 wx/wx* X *a2-m1/a2-m1 wx-844/wx*]

Only ears showing 1:1 segregation of spotted vs. colored were used to ensure that only one *En* is segregating among the progeny of the above cross.

ACKNOWLEDGEMENT

I would like to express my appreciation to Dr. Peter A. Peterson for giving me an opportunity to work in this lab, for letting me take the direction of my research in an independent direction and for his critical comments in developing this manuscript.

I would also like to thank the members of my advisory committee, Dr. J. Girton, Dr. A. Hallauer, Dr. J. Horowitz, Dr. A. Myers, and Dr. R. Thornburg for the very helpful cooperation provided until the end.

I express my appreciation to Dr. A. Atherly, Dr. D. Enger, and Ina Couture for arranging the financial assistance at a time of urgent need. I also acknowledge the financial support provided by the Pioneer Hibred International.

My very sincere appreciation to the Department of Agronomy and particularly to Ina Couture for extending help whenever needed. Many many thanks to Robbie Kerkove, Mary Johnson, and Eileen Muff for being helpful and friendly and very cooperative.

A very special thanks to Gaylan Crim, Research Associate in Maize Cytogenetics Lab for all the help, support, and friendship through out my stay and thanks for the good times.

I thank Dr. Peterson, Dr. Pat Schnable, Dr. Brian Scheffler, Dr. Andy Pereira, Jean Cormack, Elizabeth Caldwell, Dr. Young-Bao Pan, and Dr. Guru Natarajan for helping me learn maize genetics.

Thanks to all the graduate students in the lab for the camaraderie, help, good times and good fellowship and everything: They are in alphabetical order, Andy, Brian, Chen, Elizabeth, Etienne, Guru, Jayaram, Jean, Ken, Kim, Mike, Missae, Pan, Pat, Rashid, Ruying, Tom, Vijaya, and Weihong.

I also want to thank Andy, Brian, Elizabeth, Jean, Kim, Mike and Pat for sharing with me the excitement of discussing maize genetics and for many fruitful discussions on strategy and ideas of handling genetic problems.

My sincere thanks to Dr. Alfons Gierl for the inspiration and cooperation on molecular aspects of the problem.

Thanks to all the work-study people who participated in the field in the summer; all of them provided help at different times; and special thanks to Jacki, Ruth, Shari, and Shawn for good companionship.

Finally, very very sincere thanks and gratefulness to my parents, Abhimanyu and Dullabha Dash; my late grandmothers, Manika and Saria Debya; my sisters, Satyabhama, Swarnaprabha, and my brother, Sushant for the patience, encouragement, inspiration and support.